Proceedings of the 15th International Congress of Myriapodology, 18–22 July 2011, Brisbane, Australia

Edited by

Robert Mesibov & Megan Short

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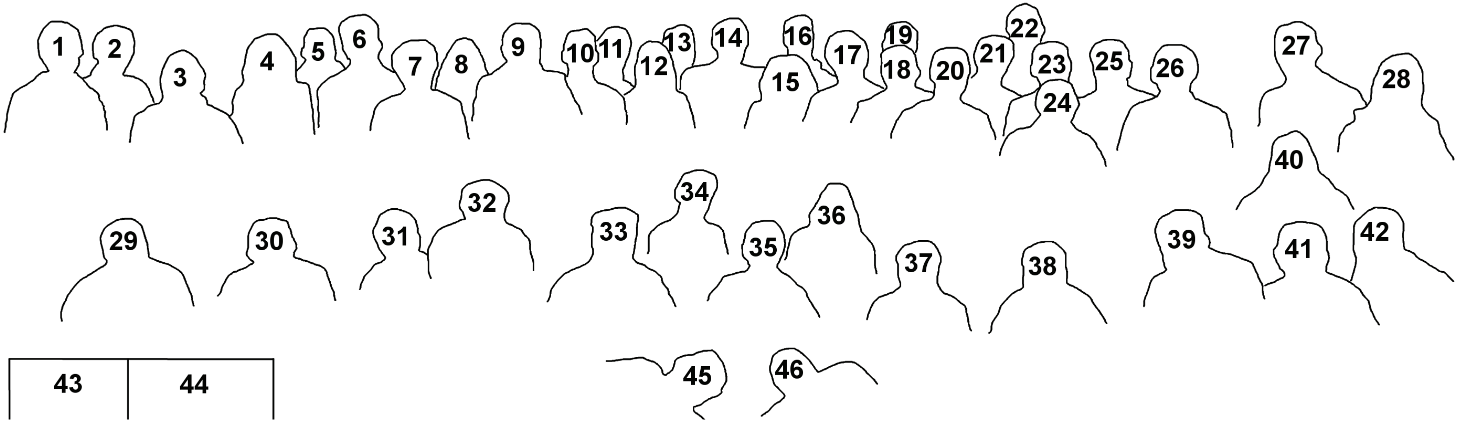
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Foreword

The papers in this special issue of ZooKeys were presented at the 15th International Congress of Myriapodology (15ICM) in Brisbane, Australia in late July 2011.

Myriapodology congresses are held every three years under the auspices of the Centre Internationale de Myriapodologie (CIM), based at the Muséum National d'Histoire Naturelle in Paris. For the past 40 years, CIM has managed a database of myriapodologists and their interests and a bibliography of currently published works. It produces an annual bulletin and maintains a website with myriapodological news and resources Thanks in large part to the CIM, the global myriapodological community is remarkably close-knit and friendly.

15ICM was the first myriapodology congress to be held in Australia and only the second in the Southern Hemisphere. The 46 participants came from 22 countries, making the 15th Congress a truly international meeting of myriapod specialists.

As organisers we are very grateful for the generous support provided by Phoenix Environmental Services of Perth, Western Australia and two Australian Government programs: the Atlas of Living Australia and the Australian Biological Resources Study.

We also thank Lyubomir Penev and the friendly staff at Pensoft Publishers for making the 15ICM papers freely available to a global online audience.

Robert Mesibov
Megan Short

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RESEARCH ARTICLE

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On some surface structures of potential taxonomic importance in families of the suborders Polydesmidea and Dalodesmidea (Polydesmida, Diplopoda)

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Abstract

Surface structures have rarely been the subject of a comprehensive study in Polydesmida despite their tremendous variety within this order. A number of these peripheral structures are here studied in most families of the suborders Polydesmidea and Dalodesmidea (sensu Hoffman 1980), using scanning electron microscopy. An illustrated description of the surface sculpture of the prozonite, the limbus and the intercalary cuticular micro-scutes on the metazonite is given for the first time for the studied families, together with an account of some other poorly known surface structures. Taken together, these characters allow us to recognize two main groupings of families. The families Ammodesmidae, Cryptodesmidae, Cyrtodesmidae, Haplodesmidae, Oniscodesmidae and Pyrgodesmidae have knobs on the posterior part of the prozonites, a toothed to lobed limbus, and no micro-scutes on the metazonites, wheras the families Fuhrmannodesmidae, Polydesmidae, Dalodesmidae, Macrosternodesmidae, Nearctodesmidae, Opisotretidae and Trichopolydesmidae have no knobs on the posterior part of the prozonites, a spiky or reduced limbus, and intercalary micro-scutes on the metazonites. The results are complemented with literature records and compared with current taxonomic and phylogenetic interpretations of the group.

Keywords

surface microsculpture, prozonite, limbus, cuticular micro-scutes, ozopore, metazonital outgrowth, taxonomy

Introduction

Whereas the gonopods have hitherto been acknowledged to be the most reliable source of characters for millipede identification, the details of external morphology have in most cases remained under-prospected. This is also true for the order Polydesmida although it is by far the most diverse millipede order in terms of non-gonopodal morphology. The taxonomy of several polydesmidan families, notably Fuhrmannodesmidae and Pyrgodesmidae, is in a deplorable state, and new taxonomic characters are badly needed. Rowe and Sierwald (2006) drew attention to the fact that the external morphology has been studied in only a few cases in millipedes, and gave an overview of the major works which dealt with this topic.

Scanning electron microscopy has, in many contemporary works, significantly helped to illustrate fine surface structures in millipedes. Mesibov (2009a) studied and described the several shapes of spiracles in species of the families Paradoxosomatidae and Dalodesmidae, demonstrated the great variation in the shape, location and density of the sphaerotrichomes on male legs in Dalodesmidae and noted the presence of different patterns in the integument sculpture of the metatergal tuberculation in two genera of the same family (see also Mesibov 2008). Moreover, the different arrangements and structures of the spinnerets in 16 families of Polydesmida were studied by Shear (2008), who delineated a notable variation of these structures within the studied taxa (see also Mesibov 2009a). The structure of the spinnerets was furthermore studied by Mesibov (2008, 2009a) who suggested a possible synapomorphy for some of the 10 studied dalodesmid genera even if recognizing that the use of spinneret structure in taxonomy of Polydesmida requires more sampling (see Mesibov 2009a).

The limbus, or posterior margin of the metazonites, was investigated by Schmidt (1962) who systematically described and compared the variation of shapes within numerous families, using only light microscopy.

Some polydesmidans of the families Cryptodesmidae, Haplodesmidae, and Pyrgodesmidae are earth-incrusted, i.e., adult specimens bear a coat of dirt. Shear (1973, 1977) described and illustrated special "boxes" and "branched tree-like setae" (Shear 1977) which supposedly keep the dirt on the cuticle.

During the study of a new pyrgodesmid species from Tunisia using scanning electron microscopy (Akkari and Enghoff 2011), we found a number of cuticular structures which have not hitherto been described. In order to assess their significance, we made a comprehensive survey of 22 species belonging to all the families of the suborders Polydesmidea and Dalodesmidea (see the list below and Table 1) except for the Dorsoporidae (Polydesmidea) and Vaalogonopodidae (Dalodesmidea) of which material was inaccessible for study.

Our study was mainly focused on three sets of characters: a) surface sculpture of the prozonite (anterior, cylindrical part of body ring); b) the limbus (posterior margin of body rings); c) intercalary micro-scutes on the surface of the metazonite. A few additional structures such as the cuticular outgrowths in earth-incrusted species and the ozopores are briefly presented below and compared. Moreover, recent literature accounts of 29 species from relevant families (see Table 2), including SEM illustrations or descriptions were checked and compared with our results.

Table 1. The studied species and the states of the three main characters examined.

	Species	**Knobs on the posterior surface of the prozonites**	**Limbus**	**Intercalary micro-scutes on metazonites**
Suborder Polydesmidea				
Ammodesmidae	*Elassystremma* sp.	+	lobes and spikes	–
Cryptodesmidae	*Aporodesmus* sp.	–	jagged lobes and spikes	–
	Elythesmus enghoffi	+	jagged lobes and spikes	–
Cyrtodesmidae	cyrtodesmid gen. sp.	+	lobes and spikes	–
Fuhrmannodesmidae	*Fuhrmannodesmus lividus*	–	reduced	+
	fuhrmannodesmid sp.	–	reduced	+
	Gyrophallus sp.	–	reduced	+
Haplodesmidae	*Prosopodesmus jacobsoni*	+	lobes	–
Macrosternodesmidae	*Ophiodesmus albonanus*	–	reduced	+
Nearctodesmidae	nearctodesmid sp.	–	reduced	+
Oniscodesmidae	*Amphitomeus attemsi*	+	lobes and spikes	–
Opisotretidae	*Corypholophus* sp.	–	reduced	?+
	Solaenaulus butteli	–	reduced	+
Polydesmidae	*Brachydesmus superus*	–	ramified spikes	+
	Propolydesmus laevidentatus	–	ramified spikes	+
Pyrgodesmidae	*Cryptocorypha ornata*	+	lobes	–
	Cynedesmus sp.	+	lobes	–
	Rharodesmus tabarkensis	+	lobes	–
	Tonodesmus sp.	+	lobes	–
Trichopolydesmidae	*Napocodesmus endogeus*	–	reduced	+
	trichopolydesmid sp.	–	reduced	+
Suborder Dalodesmidea				
Dalodesmidae	*Icosidesmus* sp.	–	ramified spikes	+

Table 2. Literature records

	Species	**Reference**
Suborder Polydesmidea		
Ammodesmidae	*Elassystremma laeve* Vandespeigel and Golovatch, 2003	VandenSpeigel and Golovatch 2003
	Elassystremma prolaeve VandenSpeigel and Golovatch, 2003	

	Species	**Reference**
Cryptodesmidae	*Aporodesmus gabonicus* (Lucas, 1858)	Schmidt 1962
	Tarmadesmus azucarensis Kraus, 1959	
Fuhrmannodesmidae	*Fuhrmannodesmus carli* Kraus, 1955	Schmidt 1962
	Salvadoria sagittalis Kraus, 1954	
	Cutervodesmus similis Kraus, 1959	
Haplodesmidae	*Agathodesmus steeli* Silvestri, 1910	Mesibov 2009b
	Cylindrodesmus hirsutus Pocock, 1889	Golovatch et al. 2001
	Eutrichodesmus armatocaudatus Golovatch et al. 2009	Golovatch et al. 2009a
	E. basalis Golovatch et al. 2009	
	E. communicans Golovatch et al. 2009	
	E. inciues Golovatch et al. 2009	
	E. similis Golovatch et al. 2009	
Oniscodesmidae	*Oncodesmoides rectus* Kraus, 1954	Schmidt 1962
Opisotretidae	*Opisotretus kraepelini* (Attems, 1907)	Schmidt 1962
Polydesmidae	*Polydesmus complanatus* (Linneaus, 1871)	Schmidt 1962
Pyrgodesmidae	*Lobiferodesmus vanuatu* Golovatch, et al., 2008	Golovatch et al. 2008
	Poratia digitata (Porat, 1889)	Adis et al. 2000
	Muyudesmus obliteratus Kraus, 1960	
	Cryptocorypha hoffmani Golovatch et al. 2011	Golovatch et al. 2011
	Myrmecodesmus hastatus (Schubart, 1945)	Bergholz et al. 2004
	Monachodesmus albus Kraus, 1958	Schmidt 1962
Suborder Dalodesmidea		
Dalodesmidae	*Ginglymodesmus tasmanianus* Mesibov 2005	Mesibov 2009c
Not assigned to any family	*Asphalidesmus bellendenkerensis* Mesibov, 2011	Mesibov 2011
	Asphalidesmus golovatchi Mesibov, 2009	
	Noteremus infimus Mesibov, 2009	Mesibov 2009c
	N. summus Mesibov, 2009	
	Procophorella innupta Mesibov, 2003	Mesibov 2003

Materials and methods

The studied material is preserved in 70 % ethanol and deposited in the Natural History Museum of Denmark (Zoological Museum, University of Copenhagen, ZMUC). Earth-incrusted specimens were cleaned for scanning electron microscopy by soaking in a solution of commercial detergent (©Biotex) and/or by ultrasound then air dried. SEM pictures were made with a JEOL JSM-6335F scanning electron microscope, then processed and assembled with Adobe Photoshop CS5 software.

When not otherwise indicated, we have followed the classification proposed by Hoffman (1980) as updated by Shelley (2003).

Studied specimens

Suborder Polydesmidea Pocock, 1887

Ammodesmidae Cook, 1896

Elassystremma sp., Tanzania, Unzungwa Mts, Iringa Region, Uzungwa Scarp Forest Res., above Chita village, 1600–1650m, 8–13.ix.1984, pitfall Traps in Montane Rain Forest, N. Scharff leg. (ZMUC 00020487).

Cryptodesmidae Karsch, 1879

Aporodesmus sp., female, Cameroun, Northwest Province, Menchum Div. Near L. Oku forest, in litter, ca. 2150m, N6°12', E10°27', 7–13.ii.1992, C. Griswold, S. Larcher, N. Scharff and C. Wanzie leg. (ZMUC 00020478).

Elythesmus enghoffi Hoffman, 1978, female, Tanzania, W. Usambara Mts, Mazumbai Forest Reserve, 19–29.ix.1992, M. Andersen leg. (ZMUC 00020477).

Cyrtodesmidae Cook, 1896

Cyrtodesmid sp., female, Colombia, Páramo de Sumapaz, soil under grasses, 3600m, 5.x.1978, H. Sturm leg. (ZMUC 00020494).

Fuhrmannodesmidae Brölemann, 1916

Fuhrmannodesmus lividus Carl, 1914, male, Colombia, Par de Monserrate, near Bogotá, 3250m, dead leaves of *Espeletia grandifolia*, 18.iv.1969, H. Sturm (ZMUC 00020483).

fuhrmannodesmid sp. (Arndt et al. 2008), female, Spain, Canary Islands, La Palma, Pared Vieja, 21.ii.-5.iii.2002, E. Arndt leg. (ZMUC 00020492).

Gyrophallus sp., female, Colombia, 1991, H. Sturm leg. (ZMUC 00020484).

Haplodesmidae Cook, 1895

Prosopodesmus jacobsoni Silvestri, 1910, female, Fiji Isl, Viti Levu Suva, in garden, 2–3.ix.1995, A van Hart leg. (ZMUC 00020476).

Macrosternodesmidae Brölemann, 1916

Ophiodesmus albonanus (Latzel, 1895), male, Denmark, NE Zaland, Copenhagen, Utterslev Mose, 22.iv.1973, H. Enghoff leg. (ZMUC 00020491).

Nearctodesmidae Chamberlin and Hoffman, 1950

nearctodesmid sp., male, Calif. Humboldt Co. Jolly Giant Canyon, 300–650m, Arcata Comm. For 13.i.1979, A.K. Johnson, R. M. Shelley leg. (ZMUC 00020482).

Oniscodesmidae de Saussure, 1860

Amphitomeus attemsi (Schubart, 1934), female, Dania: Nez UB47, Copenhagen, Botanical garden, væksthus, 16.iv.1986, H. Enghoff and Z. Korsòs leg. (ZMUC 0002046).

Opisotretidae Hoffman, 1980

Corypholophus sp., female, Thailand, Chieng Mai Province, Doi Inthanon N. P., Mae Ya, 6–700m (ZMUC 00020479).

Solaenaulus butteli (Carl, 1922), male, Fiji Isl. Viti Levu Suva, in garden, 2–3. ix.1995, A van Harten leg. (ZMUC 00020480).

Polydesmidae Leach, 1815

Brachydesmus superus (Latzel, 1884), female, Tunisia, NW, Jendouba Governorate, 9km of Hammam Bourguiba (West of Aïn Draham), N36˚48.046, E08˚39.544, 379m, Pine Forest, close to river, under stones, logs and leaf litter, 22.iii.2008, P. Stoev and N. Akkari leg. (ZMUC 00020496).

Propolydesmus laevidentatus (Loksa, 1967), male, Madeira, Faja da Nogueria, N. Side, ca. 800m.a.s.l. *Laurisilva* with *Ocoteas*, 20.xi.1980, H. Enghoff and O. Martin leg. (ZMUC 00020475).

Pyrgodesmidae Cook, 1895

Cryptocorypha ornata (Attems, 1938), unsexed fragment, Hawaii Isl., Kauai, in a grotto, moss and *Adiantum*, 20.x.1962, M. Hammer leg. (ZMUC 00020493)

Cynedesmus sp., female, La Gomera, Valle Gran Rey, litter, in banana plantation, 10m, 2.xii.1987, A. Fjellberg leg. (ZMUC 00020485).

Rharodesmus tabarkensis Akkari and Enghoff, 2011, male, Tunisia, NW, Jendouba Governorate, Tabarka, N36°58'10.5", E8°45'35.6", alt. < 40m, coastal slope below the Genoese fort, under stones, 9.iii.2009, N. Akkari and H. Enghoff leg. (ZMUC 00020532).

Tonodesmus sp., male, Spain, Almeria Sima terminal, T. M. Eidijo, 28.v.2000, M. Piquer and J.G. Pedro leg. (ZMUC-00020495).

Trichopolydesmidae Verhoeff, 1910

Napocodesmus endogeus Ceuca, 1974, female, Moldavian SSR, Tiraspol, deep in orchard soil, 1985, S. Golovatch leg. (ZMUC 00020481).

trichopolydesmid sp. Slovakia, Slovak Karst, Ardouská Cave A-04-47, 5.x.2004, A. Mock leg. (ZMUC 00020490).

Suborder Dalodesmidea Hoffman, 1980

Dalodesmidae Cook, 1896

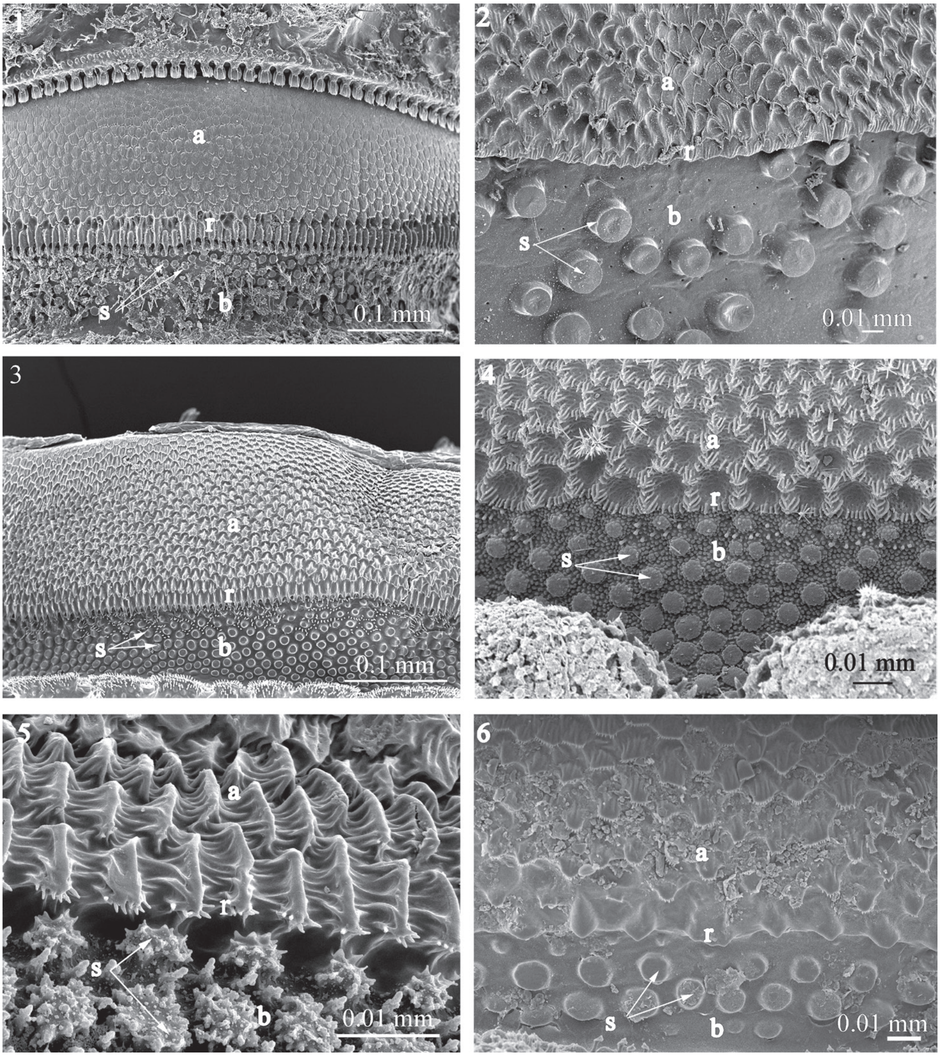
Icosidesmus sp., male, New Zealand, South Isl, Christchurch Banks Peninsula, Hinewai Reserve, Big Kanuka Trail, 3 iii.2010, S43˚48'38.0", E173˚01'15.6", 508m, sifted leaf litter and mosses, N. Scharff and G. Hormiga leg. (ZMUC 00020488).

Results

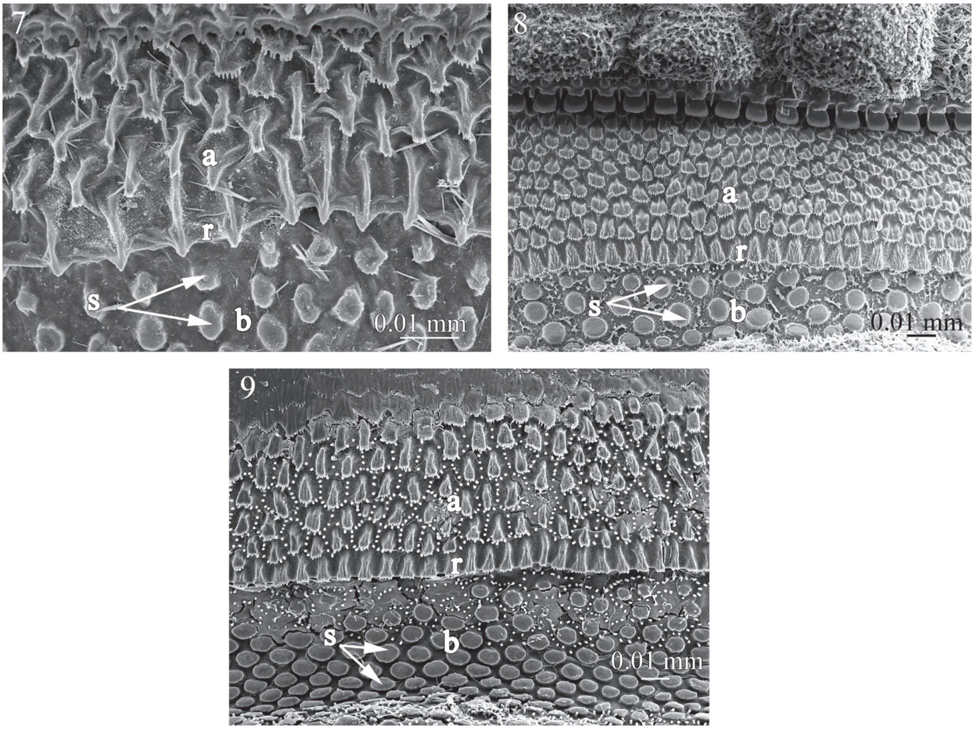
Fine sculpture of the prozonite

Figs 1–9

The prozonite of the studied species is divided into two main parts separated by a transverse ridge. While the anterior part is rather uniform, showing a scaly aspect, the posterior surface displays varied patterns within the studied families.



Figures 1–6. Fine sculpture of the prozonite in the families Ammodesmidae, Cryptodesmidae, Cyrtodesmidae, Haplodesmidae, Oniscodesmidae and Pyrgodesmidae **1** *Elassystremma* sp. **2** *Elythesmus enghoffi*, **3** cyrtodesmid sp. **4** *Prosopodesmus jacobsoni* **5** *Amphitomeus attemsi* **6** *Rharodesmus tabarkensis*. Abbreviations: **a** anterior part of the prozonite, **b** posterior part of the prozonite, **r** ridge, **s** spherical knobs.

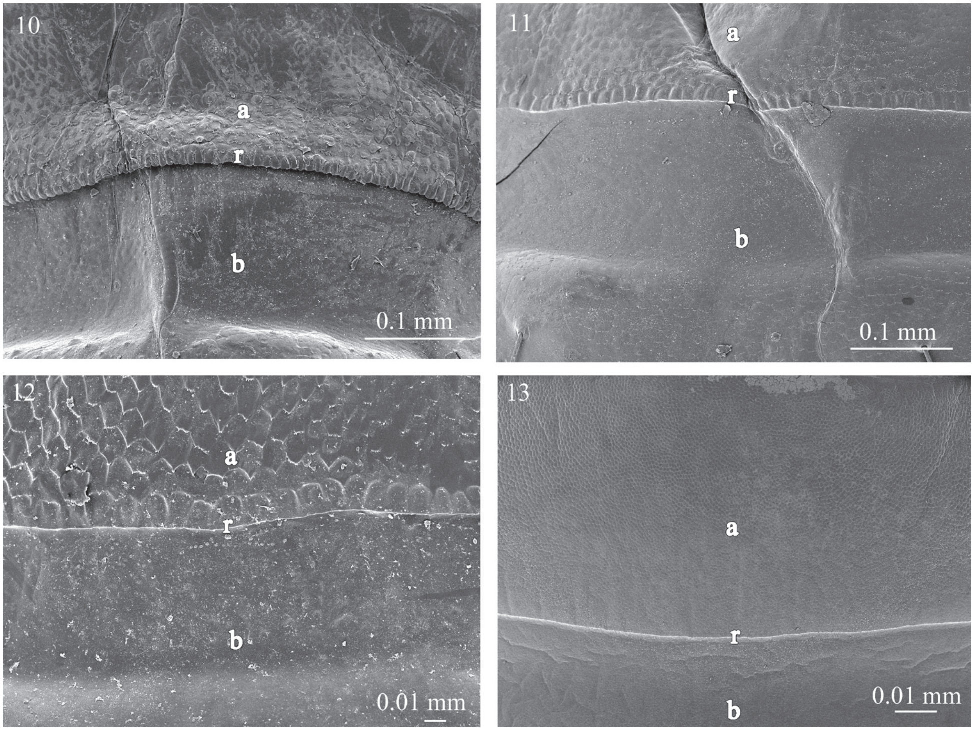


Figures 7–9. Fine sculpture of the prozonite in pyrgodesmid species. **7** *Tonodesmus* sp. **8** *Cynedesmus* sp. **9** *Cryptocorypha ornata*. Abbreviations: **a** anterior part of the prozonite, **b** posterior part of the prozonite, **r** ridge, **s** spherical knobs.

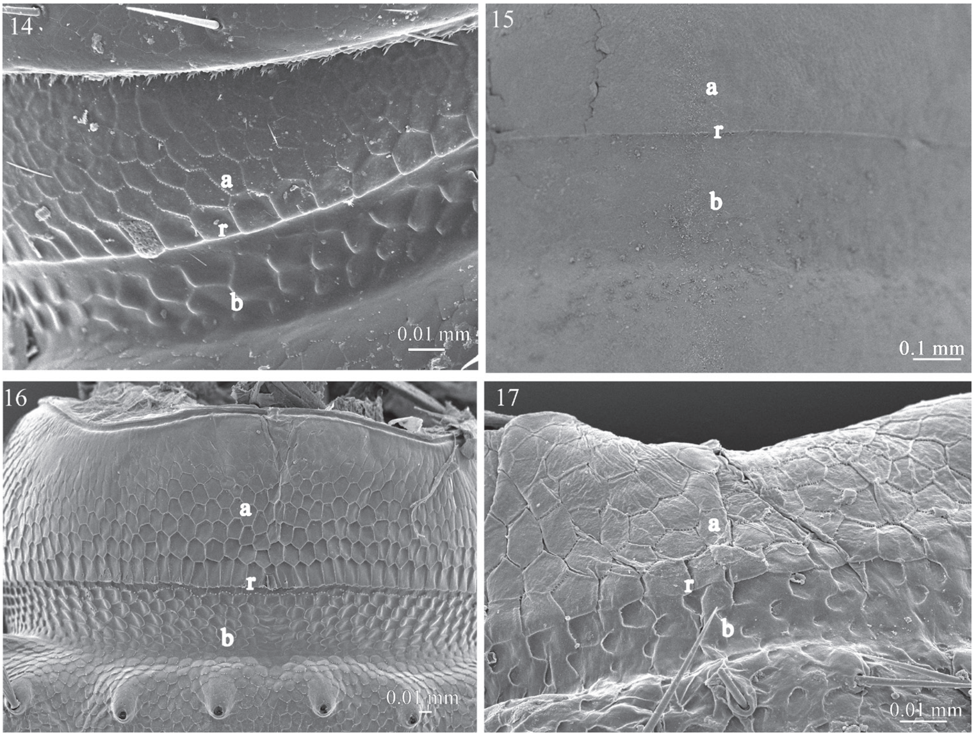
In the examined species of Ammodesmidae, Cryptodesmidae, Cyrtodesmidae, Haplodesmidae, Oniscodesmidae and Pyrgodesmidae the anterior part of the prozonite (a) displays a covering of small scaly units, juxtaposed in series of irregular transverse rows, becoming elongated in the posteriormost row to form a transverse ridge (r) marking the border of this part (Figs 1–9). In front of the ridge, the general aspect is quite uniform in all the above cited families, with lozenge-shaped units. These units could sometimes be star-like, furrowed, and marginally jagged (cyrtodesmid sp.) (Fig. 3). In *Amphitomeus attemsi* (Oniscodesmidae), the units are more elongated and strongly prominent, interconnected with parallel cuticular ridges (Fig. 5). On the other hand, the microsculpture of the anterior part of the prozonite in *Prosopodesmus jacobsoni* (Haplodesmidae) takes the shape of hollow chambers separated by walls of "microvilli-like" structures (Fig. 4). The posterior surface of the prozonite (b) is, in all examined species of these six families, characterized by a regular covering of subspherical knobs (s) placed on a smooth to microgranulated background. The cover of knobs is regularly dense in most cases (Figs 1, 3–9) though fairly dispersed in *Elythesmus enghoffi* (Cryptodesmidae) (Fig. 2). These knobs are generally uniformly smooth

(Figs 1–3, 6–9) but sometimes exhibit special configurations: lobed in *P. jacobsoni* and spiky in *A. attemsi* (Figs 4, 5).

The examined species of the families Fuhrmannodesmidae, Polydesmidae, Dalodesmidae, Macrosternodesmidae, Nearctodesmidae, Opisotretidae and Trichopolydesmidae show an anterior surface of the prozonite with polygonal units serrated marginally. However, these units are much less conspicuous and prominent than in the species of the first set of families (Figs 10–17). The posterior border of the anterior part of the prozonite is similarly marked by a transverse ridge but its units are nevertheless only slightly modified. The posterior surface of the prozonite remarkably differs by the complete absence of the sub-spherical knobs described above; instead the surface is smooth to scaly (Figs 10–17).



Figures 10–13. Fine sculpture of the prozonite in **10** *Fuhrmannodesmus lividus* (Fuhrmannodesmidae) **11** *Gyrophallus* sp (Fuhrmannodesmidae) **12** *Propolydesmus laevidentatus* (Polydesmidae) **13** *Icosidesmus* sp. (Dalodesmidae). Abbreviations: **a** anterior part of the prozonite, **b** posterior part of the prozonite, **r** ridge.



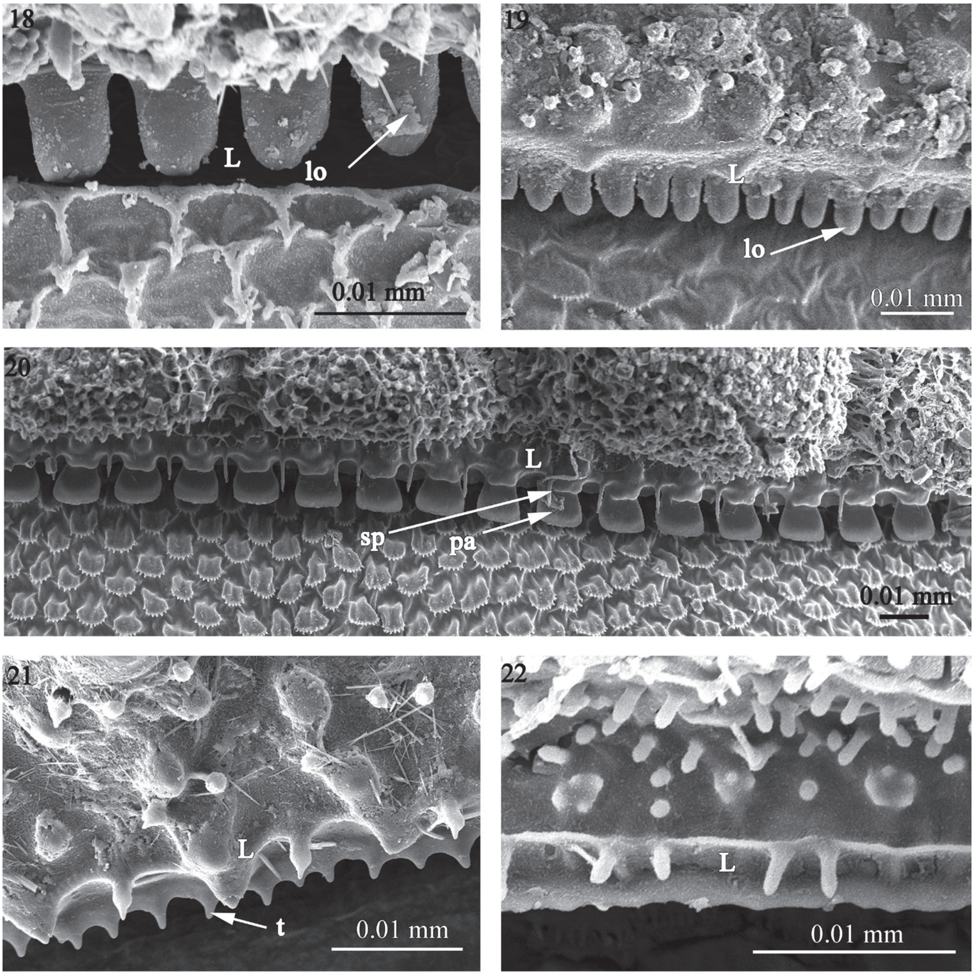
Figures 14–17. Fine sculpture of the prozonite in **14** *Ophiodesmus albonanus* (Macrosternodesmidae) **15** nearctodesmid sp. (Nearctodesmidae) **16** *Solaenaulus butteli* (Opisotretidae) **17** *Napocodesmus endogeus* (Trichopolydesmidae). Abbreviations: **a** anterior part of the prozonite, **b** posterior part of the prozonite, **r** ridge.

The limbus

Figs 18–34

The limbus (L) displays three major patterns of shapes in the studied families:

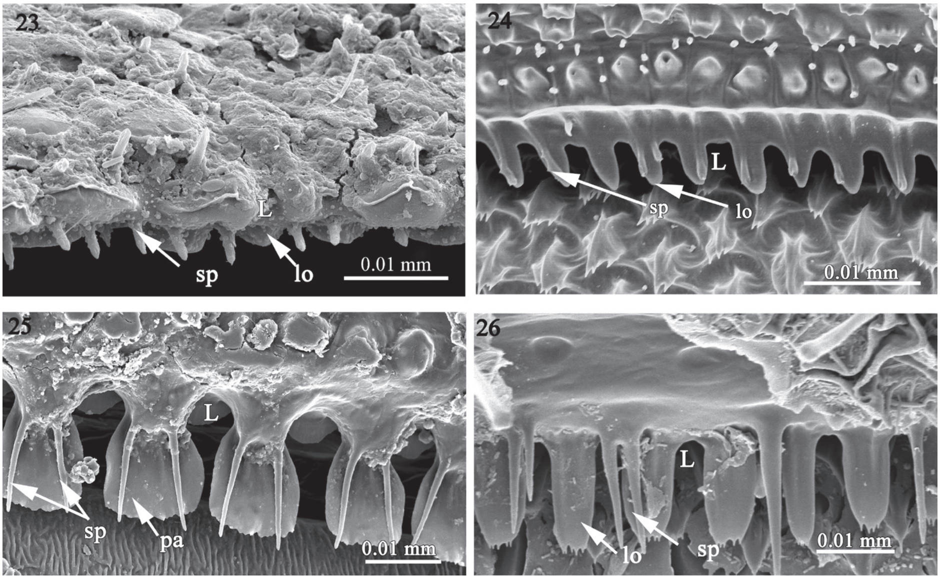
1) A regular set of rounded lobes (lo) placed in one row as in *P. jacobsoni* (Haplodesmidae), *Rharodesmus tabarkensis* (Pyrgodesmidae) (Figs 18, 19) or two superposed rows of 'palette-shaped' lobes (pa) separated by spikes (sp) as in *Cynesdesmus* sp. (Pyrgodesmidae) (Fig. 20) although in some species of the latter family (e.g. *Tonodesmus* sp. and *Cryptocorypha ornata*) the lobes are more tooth-like (t) (Figs 21, 22). The lobes are also surmounted by fine spikes as in *A. attemsi* (Oniscodesmidae), cyrtodesmid sp. (Figs 23, 24), *Elassystremma* sp. (Ammodesmidae) in which the lobes are moreover stocky or 'palette-like'(pa) and serrated (Fig. 25) and in *Elythesmus enghoffi* (Cryptodesmidae) where the spikes are more elongate and inserted between the jagged lobes (Fig. 26).



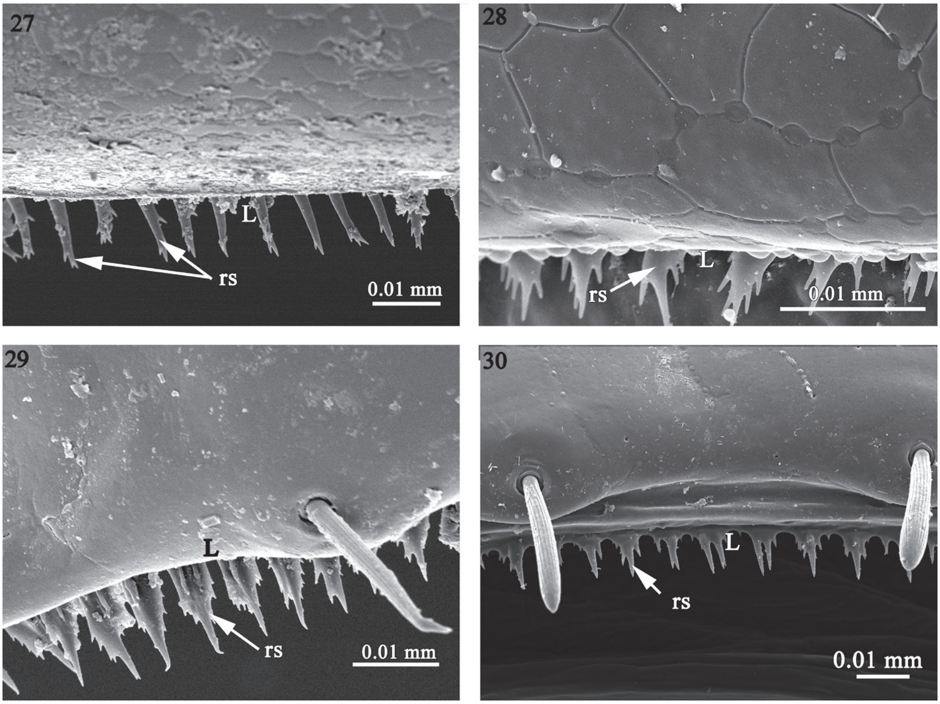
Figures 18–22. The structure of the limbus in **18** *Prosopodesmus jacobsoni* (Haplodesmidae) **19** *Rharodesmus tabarkensis* (Pyrgodesmidae) **20** *Cynedesmus* sp. (Pyrgodesmidae) **21** *Tonodesmus* sp. (Pyrgodesmidae) **22** *Cryptocorypha ornata* (Pyrgodesmidae). Abbreviations: **L** limbus, **lo** lobe, **pa** palette-like lobe, **sp** spike, **t** tooth-like lobe.

2) A series of ramified spikes (rs) in *Icosidesmus* sp. (Dalodesmidae) (Fig. 27) or "icicles" in *Ophiodesmus albonanus* (Macrosternodesmidae), *Propolydesmus laevidentatus* and *Brachydesmus superus* (Polydesmidae) (Figs 28–30).

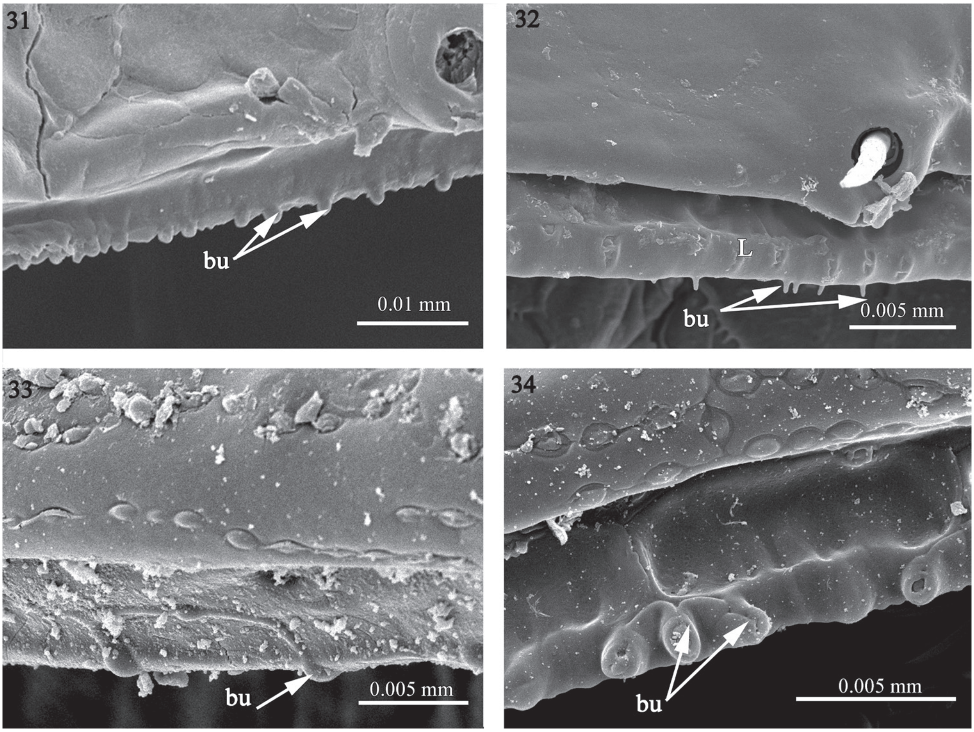
3) Reduced. In *Solaenaulus butteli* (Opisotretidae), *Napocodesmus endogeus* (Trichopolydesmidae) and *Fuhrmannodesmus lividus* (Fuhrmannodesmidae) the limbus is hardly developed, taking the shape of a regular edge bearing a few scattered bulges (bu) which could be isolated or grouped, e.g. sets of three bulges in furhmannodesmid sp. (Figs 31–34).



Figures 23–26. The structure of the limbus in **23** *Amphitomeus attemsi* (Oniscodesmidae) **24** cyrtodesmid sp. (Cyrtodesmidae) **25** *Elassystremma* sp. (Ammodesmidae) **26** *Elythesmus enghoffi* (Cryptodesmidae). Abbreviations: **L** limbus, **lo** lobe, **sp** spike, **pa**: palette-like lobe.



Figures 27–30. The structure of the limbus in **27** *Icosidesmus* sp. (Dalodesmidae), **28** *Ophiodesmus albonanus* (Macrosternodesmidae) **29** *Propolydesmus laevidentatus* (Polydesmidae) **30** *Brachydesmus superus* (Polydesmidae). Abbreviations: **L** limbus, **rs** ramified spike.

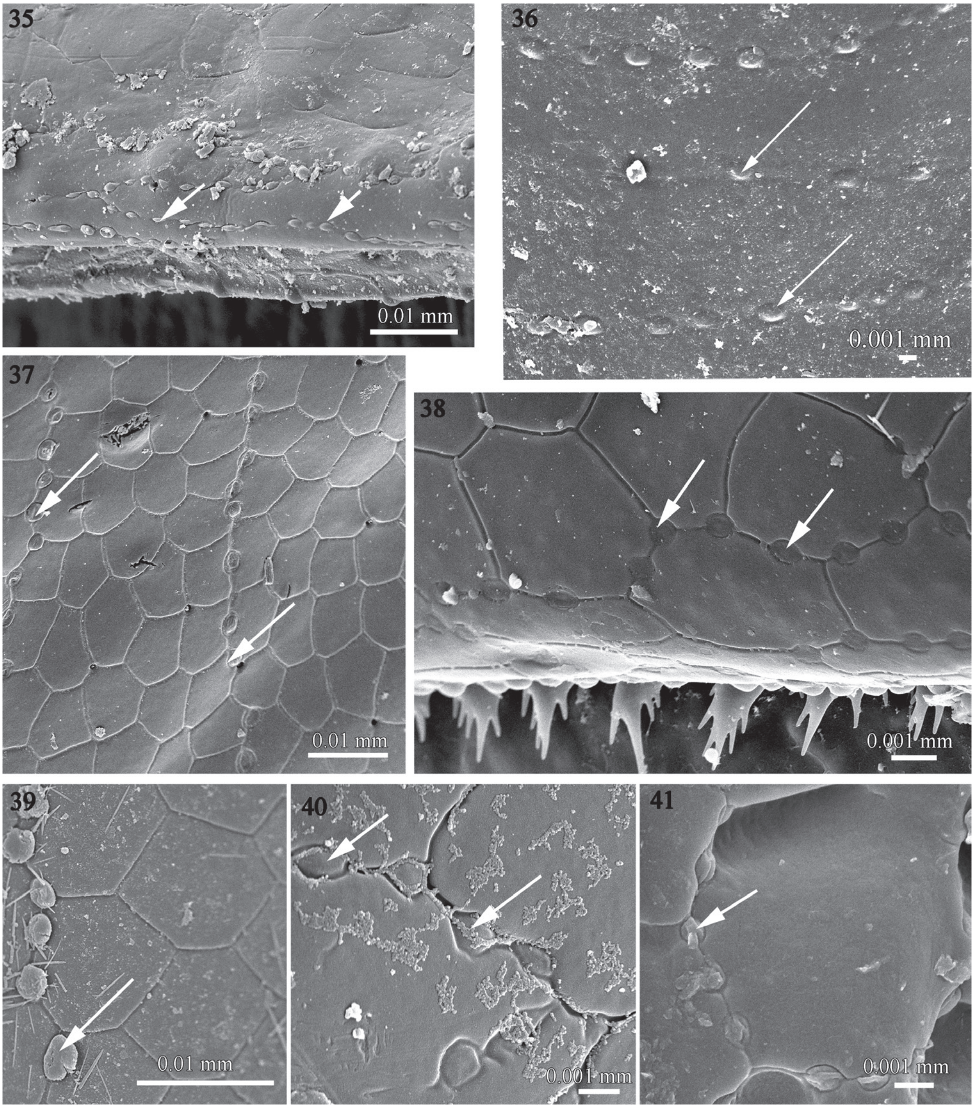


Figures 31–34. The structure of the limbus **31** *Solaenaulus butteli* (Opisotretidae) **32** *Napocodesmus endogeus* (Trichopolydesmidae), **33** *Fuhrmannodesmus lividus* (Fuhrmannodesmidae) **34** fuhrmannodesmid sp. (Fuhrmannodesmidae). Abbreviations: **bu** bulges, **L** limbus.

A peculiar structure of the metazonites: intercalary cuticular micro-scutes

Figs 35–41

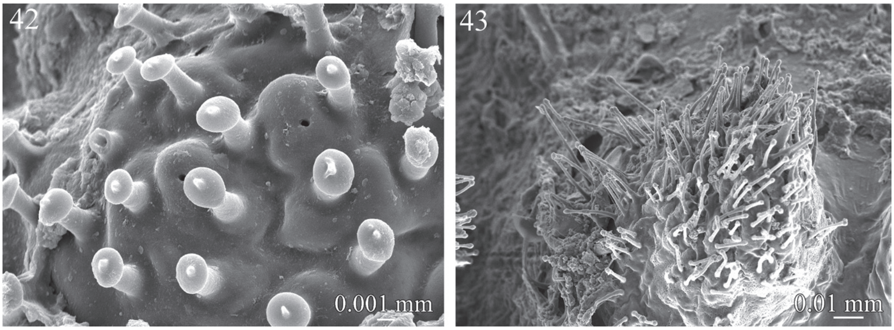
The studied species of the families Fuhrmannodesmidae, Polydesmidae, Dalodesmidae, Macrosternodesmidae, Nearctodesmidae, Opisotretidae and Trichopolydesmidae (cf. Table 1) present a peculiar structure on the metazonital surface: between the normal polygonal cuticular scutes which cover the metazonital surface there are rows of small ovoid "intercalary scutes". The placement of these structures is unlikely to be accidental or indicating any kind of bacterial infection as they seem well arranged in a regular pattern, appearing like spaced nodes or pearls aligned on strings crossing the surface of the metazonites (Figs 35–41). These structures have never been documented. However, they are visible on an illustration in Mesibov [2003, fig. 3, (right)], for *Procophorella innupta* Mesibov, 2003 (Dalodesmidea).



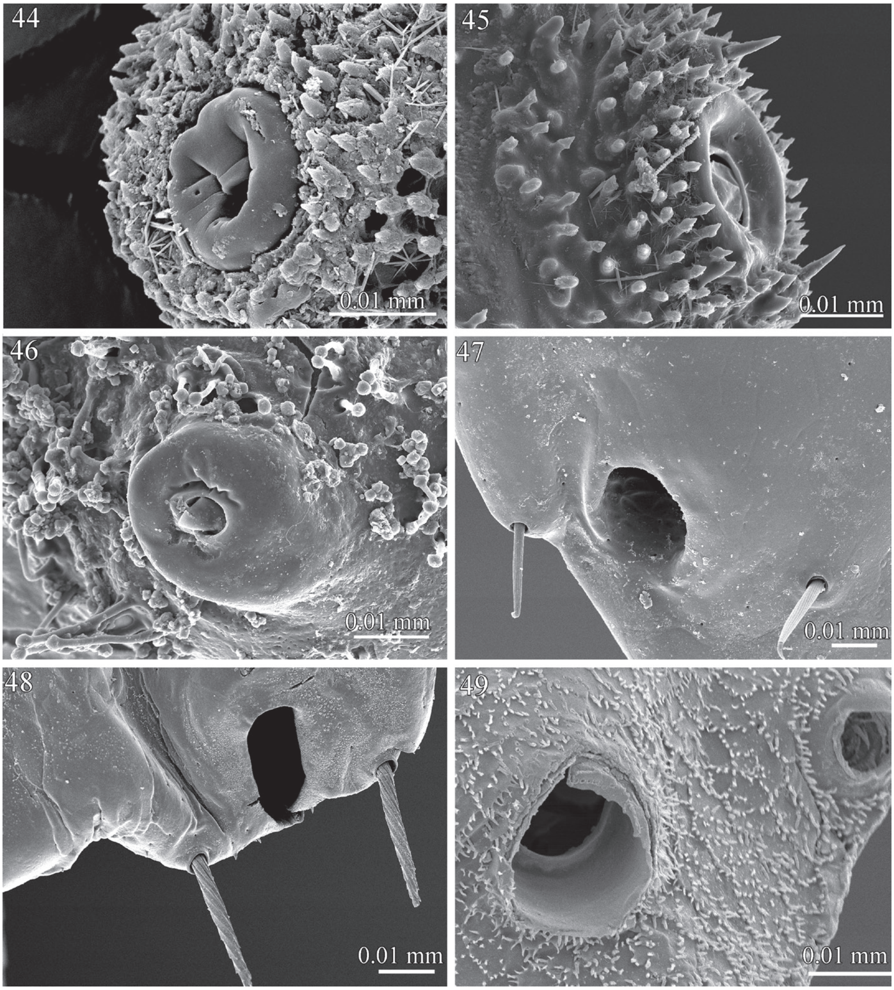
Figures 35–41. Intercalary micro-scutes on the metazonites of **35** *Fuhrmannodesmus lividus* (Fuhrmannodesmidae) **36** *Propolydesmus laevidentatus* (Polydesmidae) **37** *Icosidesmus* sp. (Dalodesmidae) **38** *Ophiodesmus albonanus* (Macrosternodesmidae) **39** nearctodesmid sp. (Nearctodesmidae) **40** *Solaenaulus butteli* (Opisotretidae) **41** *Napocodesmus endogeus* (Trichopolydesmidae). Arrows point to the micro-scutes.

Some other poorly known surface structures
Figs 42–49

The surface structure of most earth-incrusted species is characterized by the presence of papilla-like cuticular outgrowths which are particularly abundant in Ammodesmidae, Cyrtodesmidae and Pyrgodesmidae. These papillae are boletiform (mushroom-shaped)



Figures 42–43. Cuticular outgrowths **42** *Rharodesmus tabarkensis* (Pyrgodesmidae) **43** *Elassystremma* sp. (Ammodesmidae).



Figures 44–49. Ozopores **44** *Rharodesmus tabarkensis* (Pyrgodesmidae) **45** *Tonodesmus* sp. (Pyrgodesmidae) **46** *Elassystremma* sp. (Ammodesmidae) **47** *Propolydesmus laevidentatus* (Polydesmidae) **48** *Gyrophallus* sp. (Fuhrmannodesmidae) **49** *Corypholophus* sp. (Opisotretidae).

and are presumed to keep the cuticular secretions and adhering soil particles in place. Displaying variation in length, shape of the apex, and density on the surface, the papillae are short, with a rounded apex in *Rharodesmus tabarkensis* (Fig 42) and *Cynedesmus* sp., elongate and slender in *Elassystremma* sp. (Fig. 43) and in cyrtodesmid sp. The same structures have been mentioned in previous works, generally quoted as "papillis" (Silvestri 1925, 1947), "Papillen" (Attems 1940), or "microvilli" in most of Golovatch's works − illustrations can be found in Golovatch et al. (2009a, figs 24A, B, E, F). Shear (1977) was the first to provide SEM illustrations of such structures and to comment on their possible function.

The ozopores (defense gland openings) display a notable variation within the examined families. In *P. jacobsoni* (Haplodesmidae), *Tonodesmus* sp., *R. tabarkensis*, *Cynedesmus* sp. (Pyrgodesmidae) and *Elassystremma* sp. (Ammodesmidae), they open on small rounded discs, situated on the surface of the paratergites or on porosteles. The discs are of variable thickness and diameter, bear an apparent internal closing mechanism and are externally bordered with several whorls of papillae (Figs 44–46). On the other hand, the ozopores appear as simple sub-circular openings on the surface of the paratergites in *Icosidesmus* sp. (Dalodesmidae), *Propolydesmus laevidentatus* (Polydesmidae), *Fuhrmannodesmus lividus* and *Gyrophallus* sp. (Fuhrmannodesmidae), and *Corypholophus* sp. (Opisotretidae) (Figs 47–49).

Discussion

Polydesmida is the most speciose millipede order, and despite the fact that it has remained quite stable in terms of number of families (Shelley 2003), its taxonomy is far from being satisfactory. In his attempt to classify the suborders Polydesmidea and Dalodesmidea, Hoffman (1980: 146) expressed his dissatisfaction and pessimism: "The groupings set forth in the following pages are to a large extent exercises in futility, but may have some reference value in a bibliographic sense". Nevertheless, Hoffman's (1980) main classification scheme still stands and has been adopted by most subsequent authors. Simonsen (1990), studying the phylogeny of Polydesmida, made a number of changes and synonymies. Notably, Simonsen (1990) placed Dalodesmidae + Vaalogonpodidae as sister-group of Polydesmidae, i.e., the suborder Dalodesmidea nested within the suborder Polydesmidea. However, most of Simonsen's conclusions were soon after criticized by several authors because they were based on insufficient data and bold assumptions (e.g. Golovatch 1996, Shelley 2003).

Taken together, the fine sculpture of the prozonite, the structure of the limbus and the presence/absence of metazonital micro-scutes indicate two main groupings of families within the suborders Polydesmidea and Dalodesmidea. The first group (A) comprises the families Ammodesmidae, Cryptodesmidae, Cyrtodesmidae, Haplodesmidae, Oniscodesmidae and Pyrgodesmidae. These six families have a cover of subspherical knobs on the posterior surface of the prozonites (Figs 1–9) and a toothed limbus constituted of a series of lobes which may or not be surmounted by spikes (Figs 18–26).

The second group (B) encompasses the families Fuhrmannodesmidae, Polydesmidae, Dalodesmidae Macrosternodesmidae, Nearctodesmidae, Opisotretidae and Trichopolydesmidae, and is characterized by 1) an absence of knobs on the posterior surface of the prozonite which is smooth to scaly (Figs 10–17), 2) an absence of lobes on the limbus which has instead a jagged margin or spikes with varied complexity (Figs 27–34), and 3) intercalary micro-scutes on the surface of the metazonites (Figs 35–41), absent in the first set of families.

An assessment of several recent species descriptions and SEM illustrations shows in most cases similar structural arrangements of the prozonites. The presence of subspherical knobs on the posterior surface of the prozonite has been verified in a few additional genera and species of Pyrgodesmidae, such as *Lobiferodesmus vanuatu* (Golovatch et al. 2008, fig. 4C), *Poratia digitata* (Adis et al. 2000, figs 25, 26, 27; Golovatch and Sierwald 2000, figs 1, 6), *P.* (= *Muyudesmus*) *obliterata* (Adis et al. 2000, fig. 29) and *Cryptocorypha hoffmani* (Golovatch et al. 2011, fig. 38). Moreover, comparable structures are seen in the ammodesmids *Elassystremma proleave* and *E. leave* (VandenSpeigel and Golovatch 2003, figs 7, 9, 18) and for the haplodesmid *Agathodesmus steeli* (Mesibov 2009b, figs 4B, 5C, 6C). However, we noticed some differences in *Eutrichodesmus basalis* (Golovatch et al. 2009a, figs 1C, 3C), *E. armatocaudatus* (Golovatch et al 2009a fig. 6F), *E. communicans* (Golovatch et al. 2009a fig. 11F) and *E. incisus* (Golovatch et al 2009a, fig. 22E) although low image resolution doesn't allow us to draw any conclusion about these species, neither about those cited in Golovatch et al (2009b).

The fine structure of the prozonite in both species of the Tasmanian dalodesmid genus *Noteremus*, *N. summus* and *N. infimus* (Mesibov 2009c, fig. 3) perfectly agrees with what we recorded in the dalodesmid *Icosidesmus* sp. (Fig. 13). The genus *Asphalidesmus*, in contrast, exhibits a pyrgodesmid-like pattern with a conspicuous cover of sub-spherical knobs on the posterior surface of the prozonite. In his description of *A. golovatchi,* Mesibov (2009c) wrote "prozonites with narrow band of longitudinal ridges just anterior to suture, elsewhere uniformly covered with very small protuberances with blunt, rounded tips directed slightly posteriorly". Mesibov (2011) did later describe several new species of the same genus on which the posterior prozonite protuberances were visible, especially on *A. bellendenkerensis* Mesibov, 2011 (Mesibov 2011, fig. 4A).

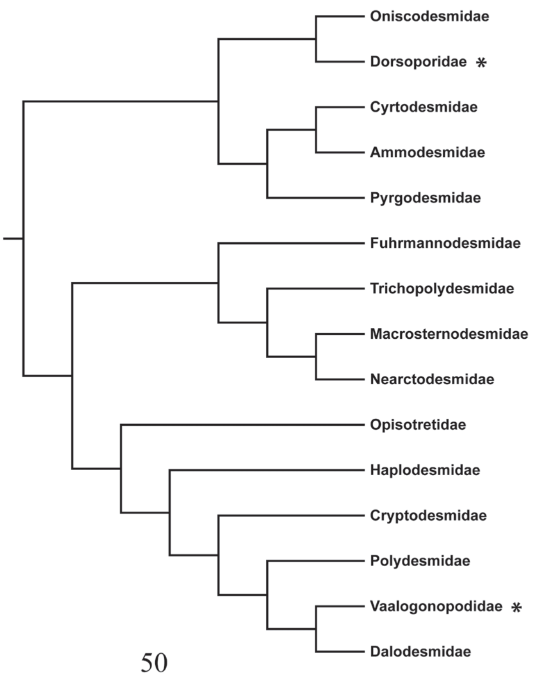
Although assigned to Dalodesmidea (Mesibov 2009c), the similarity of the genus *Asphalidesmus* to the first set of families (Ammodesmidae, Cryptodesmidae, Cyrtodesmidae, Haplodesmidae, Oniscodesmidae and Pyrgodesmidae) might not be a surprise knowing that the taxonomic position of *Asphalidesmus* has been matter of controversy. In fact, the genus was originally described in Dalodesmidae then subsequently placed in Fontariidae, Vanhoeffeniidae, and Haplodesmidae, listed in Xystodesmidae, and assigned to Cryptodesmidae and Oniscodesmidae (see Mesibov 2009c). Despite lacking sphaerotrichomes and showing a few further peripheral structures resembling more Pyrgodesmidae than Dalodesmidae and Vaalogonopodidae, *Asphalidesmus* was informally placed in the suborder Dalodesmidea (see Golovatch 2003). This was subsequently confirmed by Mesibov (2009c) although both authors agree in not assigning the genus to any family (Golovatch 2003, Mesibov 2009c).

Several literature records clearly support the patterns described above for the limbus. For example, the limbus shows a series of lobes with or without additional spikes in the pyrgodesmid species *L. vanuatu* (Golovatch et al. 2008, fig. 4D) and *Myrmecodesmus hastatus* (Bergholz et al. 2004, figs 5, 6), the ammodesmids *Elassystremma prolaeve* and *E. laeve* (VandenSpeigel and Golovatch 2003, figs 7, 18), and the haplodesmids *Agathodesmus steeli* (Mesibov 2009b, fig. 6C), *Cylindrodesmus hirsutus* (Golovatch et al. 2001, fig. 11) and *Eutrichodesmus similis* (Golovatch et al. 2009a, fig. 19E). The limbus is, on the other hand, composed of a number of ramified spikes in, for example, the dalodesmid *Ginglymodesmus tasmanianus* (Mesibov 2005, fig. 4A). Schmidt (1962, figs 22–29) provided detailed descriptions and drawings of the limbus for a number of Polydesmida species *viz. Oncodesmoides rectus* (Oniscodesmidae), *Polydesmus complanatus* (Polydesmidae), *Opisotretus kraepelini* (Opisotretidae), *Aporodesmus gabonicus, Monachodesmus albus* (Pyrgodesmidae), *Tarmadesmus azucarensis* (Cryptodesmidae), *Fuhrmannodesmus carli, Salvadoria sagittalis*, and *Cutervodesmus similis* (Fuhrmannodesmidae). In all these species, the limbus is strikingly similar to what we observed in the studied species belonging to the same families.

Though we are aware that the present data alone do not warrant a strict cladistic analysis, we have compared our findings (Fig 51) with the only existing phylogenetic work on the order Polydesmida (Simonsen 1990). The two main groups (A) and (B) mentioned above agree remarkably well with the basal dichotomy in Simonsen' cladogram (Fig 50), except for the families Haplodesmidae and Cryptodesmidae which belong to our group (A) but which according to Simonsen are in the clade which otherwise includes our group (B). However, the general knowledge about these two families and their diversity is still incomplete, and no evidence has hitherto been provided in support of their monophyly. In his revision of the Haplodesmidae, Golovatch (2009a) recognized two major 'grades', the haplodesmid and doratodesmid grades, according to their somatic (non-sexual) characters and capacity for "volvation" (coiling into a sphere). In the present study, only the haplodesmid genus *Prosopodesmus* was studied. Golovatch et al. (2009a) characterised *Prosopodesmus* as a "pyrgodesmid-like haplodesmid" and considered it as basal in the Haplodesmidae (together with *Rhipidopeltis* Miyosi, 1958).

The Cryptodesmidae studied by us alos present a complication as *Elythesmus* has knobs on the posterior part of the prozonites (Fig. 2) and a lobed limbus (Fig. 26) as in families group A, whereas *Aporodesmus* has a posterior prozonite surface free of knobs (Fig. 52) and dentate leaf-shaped (le) elements and spikes (sp) on the limbus (Fig. 53).

As stated above, the second set of families (B) have no lobes on the limbus. Instead, ramified spikes and "icicles" (Figs 27–30) can be observed in the families Dalodesmidae, Macrosternodesmidae and Polydesmidae, which clearly separates



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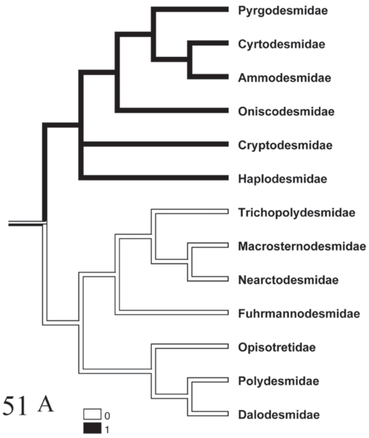
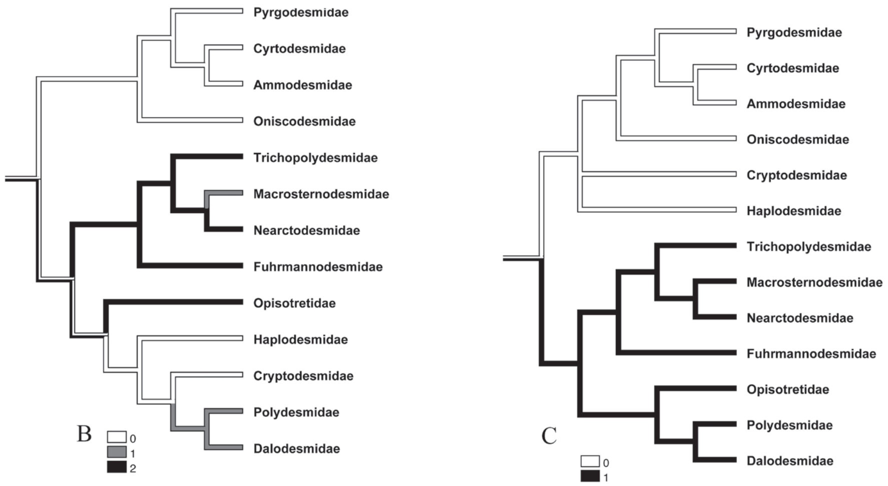
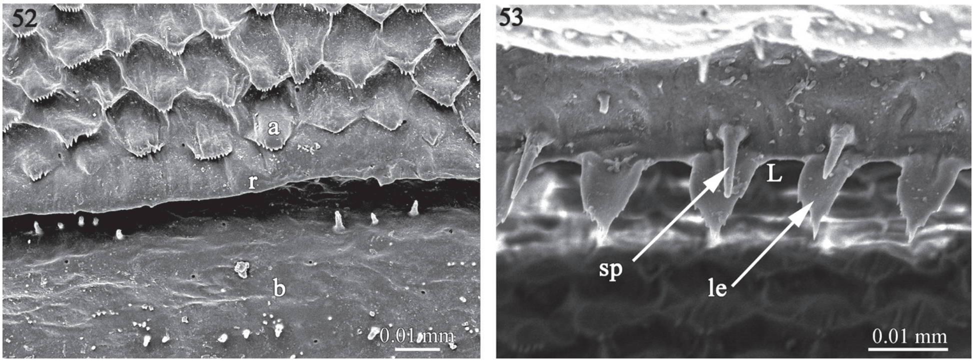




Figures 50–51. 50 Family-level cladogram of suborders Polydesmidea + Dalodesmidea according to Simonsen (1990). Haplodesmidae here corresponds to Haplodesmidae + Doratodesmidae on Simonsen's original cladogram; families not studied here are marked with asterisks **51** Branching diagrams (not cladograms) based on Fig. 50 but modified to illustrate the distribution of the different states of the three studied characters: A. presence of knobs on the anterior part of the prozonite, B. shape of the limbus, C. presence of intercalary micro-scutes on the metazonites (see Appendix for character states).



Figures 52–53 Prozonite and limbus in *Aporodesmus* sp. (Cryptodesmidae) **52** Prozonite fine sculpture **53** The limbus. Abbreviations: **a** anterior part of the prozonite, **b** posterior part of the prozonite, **L** limbus, **le** dentate leaf-shaped element of the limbus, **r** ridge, **sp** spike.

them from the Fuhrmannodesmidae, Trichopolydesmidae, Nearctodesmidae and Opisotretidae in which the limbus is hardly developed and bears but few scattered bulges (Figs 31–34). However, this separation fits neither with Hoffman's (1980) classification in which the Macrosternodesmidae, Nearctodesmidae, Trichopolydesmidae and Fuhrmannodesmidae form the superfamily Trichopolydesmoidea (see also Shelley 2003), nor with the phylogenetic analysis of Simonsen (1990) in which the Macrosternodesmidae and Nearctodesmidae appear in the same clade while the Opisotretidae, a separate clade, is grouped with a different set of families (Fig. 50). The simple limbus could well be a plesiomorphic character state expressed in the Fuhrmannodesmidae, Trichopolydesmidae, Nearctodesmidae and Opisotretidae (see also Simonsen 1990).

In the present work, we do not pretend to offer a new subordinal classification of Polydesmida, or to solve any of the taxonomic problems related to families – a colossal task which definitely will require a lot more sampling and character scoring, including the gonopod structures which haven't been considered here. However, documenting these remarkable surface structures and trying to compare them between the different families will perhaps contribute to bringing new insights, leading towards a better understanding of polydesmidean and dalodesmidean relationships.

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Appendix

List of characters and tentative character states

(1) Knobs on posterior surface of the prozonites: (0) absent; (1) present

(2) Shape of the limbus: (0) with one or two rows lobes additionally bearing simple spikes; (1) with ramified spikes and icicles; (2) reduced

(3) Rows of small intercalary scutes on metazonites: (0) absent; (1): present

Character matrix

Only the species studied by us have been coded.

	Char. no.⟶	**1**	**2**	**3**
Suborder Polydesmidea Pocock, 1887				
Ammodesmidae Cook, 1896	*Elassystremma* sp.	1	0	0
Cryptodesmidae Karsch, 1879	*Elythesmus enghoffi* Hoffman, 1978	1	0	0
	Aporodesmus sp.	0	0	0
Cyrtodesmidae Cook, 1896	cyrtodesmid sp.	1	0	0
Fuhrmannodesmidae Brölemann, 1916	*Fuhrmannodesmus lividus* Carl, 1914	0	2	1
	fuhrmannodesmid sp.	0	2	1
	Gyrophallus sp.	0	2	1
Haplodesmidae Cook, 1895	*Prosopodesmus jacobsoni* Silvestri, 1910	1	0	0
Macrosternodesmidae Brölemann, 1916	*Ophiodesmus albonanus* (Latzel, 1895)	0	1	1
Nearctodesmidae Chamberlin and Hoffman, 1950	nearctodesmid sp.	0	2	1
Oniscodesmidae de Saussure, 1860	*Amphitomeus attemsi* (Schubart, 1934)	1	0	0
Opisotretidae Hoffman, 1980	*Corypholophus* sp.	0	2	?+
	Solaenaulus butteli (Carl, 1922)	0	2	+
Polydesmidae Leach, 1815	*Brachydesmus superus* (Latzel, 1884)	0	1	1
	Propolydesmus laevidentatus (Loksa, 1967)	0	1	1
Pyrgodesmidae Cook, 1895	*Rharodesmus tabarkensis* Akkari and Enghoff, 2011	1	0	0
	Tonodesmus sp.	1	0	0
	Cynedesmus sp.	1	0	0
	Cryptocorypha ornata (Attems, 1938)	1	0	0
Trichopolydesmidae Verhoeff, 1910	trichopolydesmid. sp.	0	2	1
	Napocodesmus endogeus Ceuca, 1974	0	2	1
Suborder Dalodesmidea Hoffman, 1980				
Dalodesmidae Cook, 1896	*Icosidesmus* sp.	0	1	1

Char. no.⟶	**1**	**2**	**3**
Ammodesmidae	1	0	0
Cryptodesmidae	1	0	0
Cyrtodesmidae	1	0	0
Fuhrmannodesmidae	0	2	1
Haplodesmidae	1	0	0

Char. no.⟶	**1**	**2**	**3**
Macrosternodesmidae	0	1	1
Nearctodesmidae	0	2	1
Oniscodesmidae	1	0	0
Opisotretidae	0	2	1
Polydesmidae	0	1	1
Pyrgodesmidae	1	0	0
Trichopolydesmidae	0	2	1
Dalodesmidae	0	1	1

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A revision of the millipede genus *Riukiupeltis* Verhoeff, 1939 (Diplopoda, Polydesmida, Paradoxosomatidae), with comments on the status of related species

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Abstract

The East Asian millipede genus *Riukiupeltis* Verhoeff, 1939 is revised, and is restricted to a single species, *R. jamashinai* Verhoeff, 1939. Examination of the type specimens and freshly collected material from the Ryukyu Archipelago and Vietnam show that both subsequently allocated species, *Riukiupeltis uenoi* Murakami, 1975, and *R. falcatus* (originally *Haplogonosoma falcatum* Attems, 1953, reallocated by Jeekel 1968), do not belong to this genus; moreover, they are not even congeneric with each other. According to our morphological observations, including the gonopods, *R. uenoi* is closer to the widespread *Chamberlinius hualienensis* Wang, 1956, hence we propose the new combination *Ch. uenoi* (Murakami, 1975), **comb. n.** *Riukiupeltis falcatus*, on the other hand, represents a separate, as yet monotypic, genus *Simplogonomorpha* **gen. n.**, distinct both from *Haplogonosoma* Brölemann, 1916 *sensu* Golovatch et al. (1995), and from Verhoeff's original *Riukiupeltis*. Additionally, *Simplogonomorpha falcata* (Attems, 1953), **comb. n** is re-described here based on fresh material from Vietnam. A key and colour habitus-illustrations to all three species are also provided here.

Keywords

Revision, *Riukiupeltis*, Paradoxosomatidae, Polydesmida, Vietnam, Japan

Introduction

The millipede genus *Riukiupeltis* was established for a single species *R. jamashinai* by Verhoeff, 1939 from the Ryukyu island Miyako, Japan (Riukiu and Mijako, in German). Jeekel (1968) referred to the genus in his monograph on the distribution of the family Paradoxosomatidae, and placed it in the tribe Tonkinosomatini. Moreover, he also tentatively allocated the species *Haplogonosoma falcatum* Attems, 1953, described from Xieng Khoang, Laos, to *Riukiupeltis*. Murakami (1975) described a new species *Riukiupeltis uenoi* from Sabichi-go Cave, Ishigaki-jima island, the Ryukyus. Therefore, altogether three species have been assigned to *Riukiupeltis*. The genus belongs to the tribe Chamberlinini, together with *Aponedyopus* Verhoeff, 1939, *Chamberlinius* Wang, 1956, *Geniculodesmus* Chen, Golovatch and Chang, 2008, and *Haplogonosoma* Brölemann, 1916 (Chen et al. 2011).

Following several discussions on the genus in the past (Jeekel 1968, Hoffman 1973, Murakami 1975, Chen et al. 2011), its status is still dubious, so our purpose here is to provide a revision of the genus based on fresh material and type specimens.

Material and methods

Fresh material of *Riukiupeltis falcatus* (Attems, 1953) and *R. uenoi* Murakami, 1975 was collected from Bi Doup National Park, Lam Dong province, Vietnam, and Iriomote-jima and Ishigaki-jima islands, the Ryukyu Archipelago, Japan, respectively. The type specimen of *R. jamashinai* was studied as a loan from the Bavarian State Collection of Zoology, Munich, Germany (BSCZ), whereas the holotype of *R. uenoi* was borrowed from the National Museum of Nature and Science, Tokyo (NMNS). Further material is shared between the University Museum (Fujukan) of the University of the Ryukyus, Okinawa (RUMF), the Hungarian Natural History Museum, Budapest (HNHM), and the Institute of Ecology and Biological Resources (IEBR), Hanoi, Vietnam. In addition, new material of *R. jamashinai* was identified in the collection by M. Shimojana, acquired in 1979 on Miyako-jima island.

Line drawings were made by using an Olympus SZX10 (ADN), and a Leica M125 (ZK) stereo microscope with drawing tube attached. SEM images were made by using a Hitachi S4800 scanning electron microscope. Colour photographs were taken by ZK using a Nikon D90 digital camera with macro lens and Leica microscope photo tube attached. The distribution map was generated using the software DIVA-GIS version 7.0.

Taxonomic account

Riukiupeltis Verhoeff, 1939

http://species-id.net/wiki/Riukiupeltis

Riukiupeltis Verhoeff, 1939: Zoologischer Anzeiger 127 (5/6): 121–125 Type species: *Riukiupeltis jamashinai* Verhoeff, 1939, by monotypy
Riukiupeltis:– Attems 1940: Das Tierreich 70: 546–547.
Riukiupeltis:– Takakuwa 1954: *[Diplopoda of Japan]*: 51.
Riukiupeltis:– Jeekel 1968: *On the classification and geographical distribution of the family Paradoxosomatidae (Diplopoda, Polydesmida)*, Nederlandse Entomologische Vereiniging: 62, 76. (placed in the tribe Tonkinosomatini)

Diagnosis. Gonofemorite strongly curved, distal part somewhat swollen and membraneous. Postfemoral region demarcated from femorite by obvious cingulum, and bent continuously forming almost a complete circle with femorite. Postfemoral regions consisting of a thick, strong and free solenomere, and an extremely short solenophore (= tibiotarsus).

Type species. *Riukiupeltis jamashinai* Verhoeff, 1939

Remarks. This genus is relatively close to the genus *Chamberlinius* Wang, 1956, however, it definitely differs in gonopod conformation: femorite without lamina; solenophore very short, thick, and without any basal processes.

Riukiupeltis jamashinai Verhoeff, 1939

http://species-id.net/wiki/Riukiupeltis_jamashinai
Figs 1–2, 6A, 7A, 8A, Map 1

Riukiupeltis jamashinai Verhoeff, 1939: Zoologischer Anzeiger, 127 (5/6): 125, fig.8–9.
Riukiupeltis jamashinai:– Attems 1940: Das Tierreich 70: 547, fig. 693.
Riukiupeltis jamashinai:– Takakuwa 1954: *[Diplopoda of Japan],* 52, figs 51–52.
Riukiupeltis jamashinai:– Jeekel 1968: *On the classification and geographical distribution of the family Paradoxosomatidae (Diplopoda, Polydesmida)*, Nederlandse Entomologische Vereiniging: 76.
Riukiupeltis jamashinai:– Nakamura and Korsós 2010: Acta Arachnologica 59(2): 82.

Material studied. Holotype male, in fragments – only 11 segments in 5 pieces – with segments around gonopods missing, Reg.-Nr. ZSMA20052252, and two slides with gonopods, Reg.-Nr. ZSMA20035204, and legpairs 1–7, Reg.-Nr. ZSMA20035205 (all BSCZ).

Figure 1. *Riukiupeltis jamashinai* Verhoeff, 1939, right gonopod preparation of holotype (slide Reg.-Nr. ZSMA20035204) (red arrow marks solenophore)

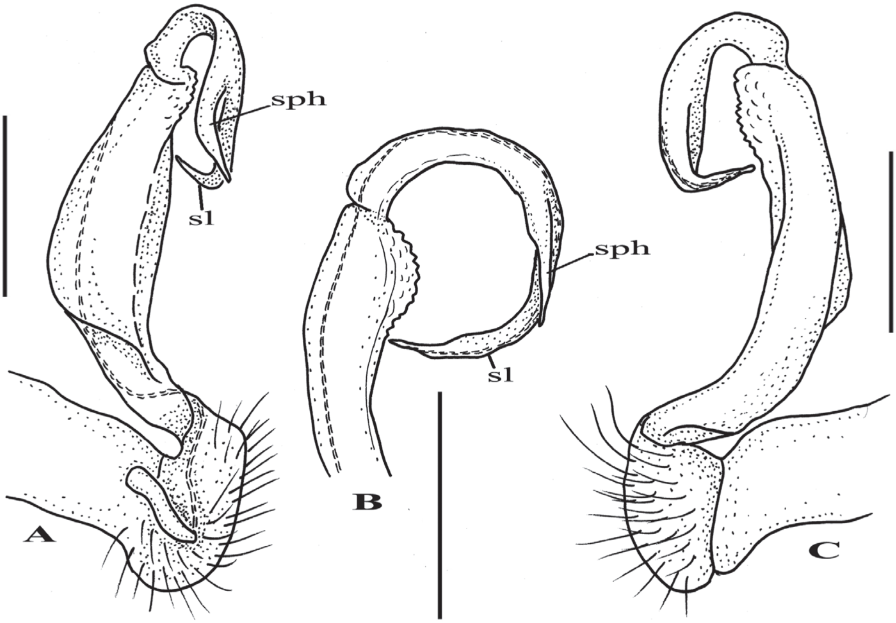


Figure 2. *Riukiupeltis jamashinai* Verhoeff, 1939, left gonopod of male from Fukumine-no-kara cave, Miyako-jima Island, 25 August 1979, leg. M. Shimojana **A** mesal view **B** dorsal view **C** ventro-lateral view (*sl* = solenomere, *sph* = solenophore or tibiotarsus). Scale bars = 0.5 mm.

New records: 2 males, 2 females, Japan, Ryukyu Archipelago, Miyako-jima Island, Rinko-abu (cave), 21 August 1979; 8 males, 2 females, Japan, Ryukyu Archipelago, Miyako-jima Island, Fukumine-no-kara (cave), 25 August 1979; 1 male, 1 female, 1 juv., 1 fragment, Japan, Ryukyu Archipelago, Miyako-jima Island, Nishibe zuzaga (cave), 26 August 1979, all leg. M. Shimojana (in the collection of M. Shimojana, Okinawa).

Distribution. Japan, Ryukyu Archipelago, Miyako-jima island.

Remarks. Although after the description of *R. jamashinai* in 1939, Jeekel (1968) and Hoffman (1973) commented that gonopod tibiotarsus is missing in this species, Verhoeff's line drawing clearly shows it as depicted from the slide preparation (Fig. 1). Re-examining the type specimen and the slide of the gonopod, as well as studying newly identified specimens found in Shimojana's collection, we are able to confirm that a gonopod tibiotarsus (=solenophore, ***sph*** in Fig. 2) is present, although it is small and closely attached to solenomere (***sl*** in Fig. 2).

Chamberlinius uenoi (Murakami, 1975), comb. n.

http://species-id.net/wiki/Chamberlinius_uenoi
Figs 3, 6C, 7C, 8C, Map 1

Riukiupeltis uenoi Murakami, 1975: Bulletin of the National Science Museum, Tokyo, {A}1(2): 105–107, fig. 9

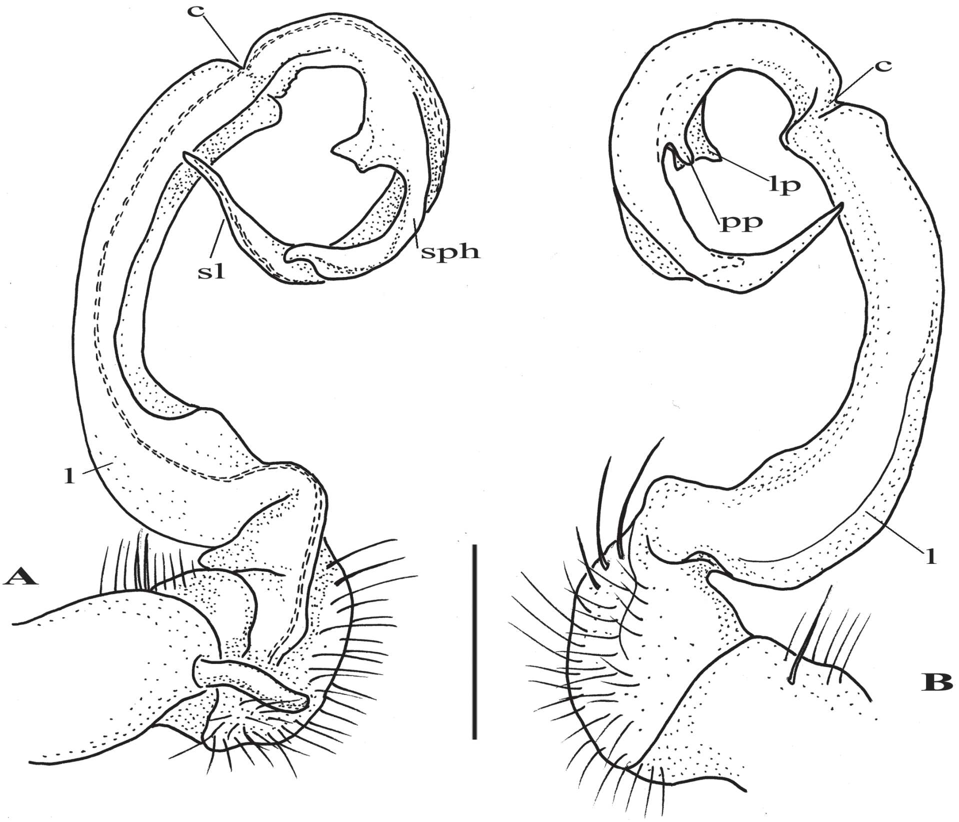


Figure 3. *Chamberlinius uenoi* (Murakami, 1975) comb. n.**,** left gonopod of male Mt. Banna-dake, Ishigaki-jima Island **A** dorso-mesal view **B** lateral view. (*l* = lamina, *c* = cingulum, *sl* = solenomere, *sph* = solenophore or tibiotarsal process, *lp* = laminar process, *pp* = pointed process)). Scale bar = 0.5 mm.

Riukiupeltis uenoi:– Nakamura and Korsós 2010: Acta Arachnologica 59(2): 82

Material studied. Holotype male, NSMT-My 358, "Japan, Okinawa Pref., Is. Ishigaki-jima, Ibaruma, Sabichi-go Cave, 31 July 1973, coll. by S. Uéno" (NMNS).

New records: 2 males, 1 female, Japan, Ryukyu Archipelago, Yaeyama Island Group, Ishigaki-jima Island, Banna-dake, secondary forest, N24.3859°–E124.1651°, 30 August 2009, leg. Z. Korsós & Y. Nakamura (RUMF); 1 male, 2 females, Japan, Southern Ryukyus, Yaeyama Group, Iriomote-jima Island, Funaura, around university research station, N24.3929°, E123.7913°, secondary forest, 18 January 2011, leg. Z. Korsós (RUMF); 1 male, 1 female, Japan, Southern Ryukyus, Yaeyama Group, Iriomote-jima Island, Mihara, along Aira river, primary forest, N24.3400°, E123.9137°, in decaying log, 12 m a.s.l., 19 January 2011, leg. Z. Korsós (HNHM).

Distribution. Japan, Ryukyu Archipelago, Yaeyama Group, Ishigaki-jima and Iriomote-jima islands.

Remarks. Murakami (1975) when describing the species commented that the terminal portion of the gonopod is more complicated than that of *Riukiupeltis jamashinai* Verhoeff, 1939. He also agreed with Verhoeff, 1939 in its configuration, and placed his species in *Riukiupeltis*. However, the species *uenoi*, in fact, differs largely from the type species *R. jamashinai* in gonopod conformation.

After studying the type and freshly collected specimens, it became clear that the species *uenoi* is strongly different from *Riukiupeltis jamashinai* in its gonofemorite having a long lamina ***l***, and a longer solenophore with basal processes ***pp*** and ***lp*** (Fig 3). We found that this species is more similar to *Chamberlinius hualienensis* Wang, 1956. Both *Ch. hualienensis* and *Ch. uenoi* comb. n. have well-developed paraterga (Figs 7A–B), large, slender and strongly concave gonofemorite, with a lamina at the mesal side (***l*** in Fig. 3). Postfemoral region is demarcated from femorite by obvious cingulum (***c*** in Fig. 3), and includes a long and large solenomere (***sl***) reaching femur, and a shorter solenophore (***sph***) with a basal lobe. However, the two species differ from each other in the length of the postfemoral processes, by the shape of the small basal processes on the solenophore, and by live colouration. The dark brown, transversal metatergal bands in *Ch. uenoi* comb. n. are not divided by a median light brown longitudinal line (Figs 7C, 8C) as in *Ch. hualienensis* (Figs 7B, 8B). Moreover, *Ch. uenoi* comb. n. is strictly confined to undisturbed, natural evergreen broadleaf forests, and can only be found deep in decaying dead wood, whereas *Ch. hualienensis* has a strong tendency for being synanthropic, and dispersed in large numbers onto many islands (especially in the southern part of Japan) by human activities.

Simplogonomorpha gen. n.

urn:lsid:zoobank.org:act:878B9422-C9BB-462E-87A5-4A2610BB5B52
http://species-id.net/wiki/Simplogonomorpha

Type species. *Haplogonosoma falcatum* Attems, 1953, by present designation

Diagnosis. This genus, *Simplogonomorpha* gen. n. can be distinguished from other genera within the tribe Chamberlinini Wang, 1956 (as defined by Chen et al. 2011) by the following characters: paraterga modestly developed, gonopod very simple, gonotelopodite tapering and distally curved down as much as forming U-shape or an almost complete circle. Solenomere very simple, thick, but slender, and strongly curved down. Solenophore (= gonopod tibiotarsus) absent.

Etymology. A feminine noun to emphasize the simple gonopod conformation.

Remarks. Jeekel (1968) in his classification of the family Paradoxosomatidae stated that "It appears that in *Riukiupeltis* the gonopod tiobiotarsus is also completely lost, although Verhoeff was of a different opinion when he described *jamashinai*". He believed that the gonopod tibiotarsus (= solenophore) was lost in *Riukiupeltis,* so he transferred Attems's species *Haplogonosoma falcatum* to this genus.

In fact, the solenophore of *R. jamashinai* still exists, although short, and somewhat hidden next to the solenomere, whereas solenophore of *Simplogonomorpha* gen. n. is totally missing. A comparison of genera in the tribe Chamberlinini is provided in Table 1.

Table 1. Comparison of genera of Chamberlinini

No.	Characters	*Riukiupeltis*	*Chamberlinius*	*Simplogonomorpha*	*Aponedyopus*	*Haplogonosoma*	*Geniculodesmus*
1	Paraterga	Well-developed	Well-developed	Modestly-developed	Poorly-developed	Poorly-developed	Poorly-developed
2	Sternal process between coxae 4	Missing	Missing	Two separate cones	A single or bifid process	A single process	Linguiform
Gonopod conformation							
3	Coxa	Long, cylindrical but not thick	Long, thick and subcylindrical	Thick and subcylindrical	Long and subcylindrical	Long and subcylindrical	Long and subcylindrical
4	Femorite	Long and slender, with a rugose membranous lamina at distal part of lateral side	Especially long, slender, simple without any outgrowths, however, sometimes, with a lamina at mesal side	Especially long, but simple and slender, devoid of any lobes or lamina.	Long, but broadened parabasally at dorsal side, without any lamina or processes	Long, slender and erected, a little bit broadened distally, devoid of any modifications	Long, slightly curved, not broadened at base
5	Postfemoral part	Demarcated by cingulum	Demarcated by cingulum	Demarcated by cingulum	Demarcated by obvious sulcus at lateral side	Set off by oblique sulcus at lateral side, apical part with a big tooth.	absent
6	Solenomere	Long, thick and simple, not sheathed by solenophore	Long, thick and simple, not sheathed by solenophore	Thick, but slender, and strongly curved downwards	Flagiliform, almost completely sheathed by solenophore, sometimes, only tip exposed	Long, slender and coiled, subfiliform	Long, flagiliform, almost sheathed by solenophore
7	Solenophore	Very short, simple, without any additional processes	As long as solenomere, with a basal dentiform outgrowth	Completely missing	Base with a small, obvious lobe. Tip with bifid lobe	Laminate, coiled, and as long as solenomere	Long and coiled, with a process at base

Simplogonomorpha falcata **(Attems, 1953), comb. n.**

http://species-id.net/wiki/Simplogonomorpha_falcata
Figs 4–5, 7D, Map. 1

Haplogonosoma falcatum Attems, 1953: Mémoires du Muséum National d'Histoire Naturelle, Paris {N. S., Sér. A, Zool.}, 5(3): 177, figs 81–82.

Riukiupeltis falcatus:– Jeekel 1968: *On the classification and geographical distribution of the family Paradoxosomatidae (Diplopoda, Polydesmida)*, Nederlandse Entomologische Vereiniging: 62.

Material studied. 3 males, 2 females, (IEBR-166), Vietnam, Lam Dong prov., Bi Doup-Nui Ba National Park, corn field, 1400m a.s.l., pitfall traps, 2–9 April 2008, leg. Anh. D. Nguyen; 1 male, 1 female, 1 juvenile, (IEBR-167), same locality, grasslands, 1400m a.s.l., pitfall traps, 2–9 April 2008, leg. Anh. D. Nguyen; 1 female, (IEBR-168), same locality, bushes near stream, 1400m a.s.l., pitfall traps, 25 April 2008, leg. Anh. D. Nguyen; 4 males, 2 females, (IEBR-169), same locality, evergreen forest, 1800m a.s.l., 25 March–23 April 2008, leg. Anh. D. Nguyen;1 male (IEBR-125), Vietnam, Khanh Hoa Prov., Hon Ba Mts., 1300–1500m a.s.l., primary forest, 15–24 April 2006, leg. Anh. D. Nguyen; 1 male, 1 female, (HNHM), same data as sample IEBR-125.

Description. *Head*: yellowish-brown to blackish brown, a slightly paler toward labrum. Epicranial suture distinct, obviously deep.

Antennae: short and stout, yellowish-brown to blackish brown. Length of antennomere 2 subequal to that of antennomere 3, 4 or 5. Antennomere 6 shorter and claviform.

Body: yellowish-brown to blackish brown on terga, paler on paraterga and pleura. Body parallel-sided on somites 5–17, thereafter gradually tapering.

Surface of metaterga general fine in posterior part, but with small oblique or longitudinal rugulose in anteriormost part. Stricture dividing pro- and metazona deep, obvious and beaded. Prozona surface shagreened with fine microgranulation**.**

Transverse sulcus on metaterga starting from somite 5 and more evident on subsequent somites. Metaterga with a row of 2+2 setae in pre-sulcus part. Axial line vague.

Paraterga not well-developed, small, look like small keels in poreless segments, but more developed in pore-bearing somites. Ozopore located on lateral side, near tip of angular paraterga of segments 5, 7, 9–10, 12–13 and 15–19.

Pleura shagreened with fine microgranulation. Pleurosternal carinae rather well-developed in anteriormost segments, gradually decreasing posteriorly.

Epiproct truncated and curved down ventrad, with 4 strong setae on tip. Anal valves sub-semicircular with 1+1 long setae, and a deep emargination inbetween. Hypoproct trapeziform, with 1+1 setae.

Legs: yellow to yellowish brown, short and stout. Tarsal brushes present until legpair 10, sparsely until legpair 16, and completely missing thereafter.

Sterna: normal, sparsely setose, with two large tubercles between coxae 4.

Male gonopod: very simple, hook-like in dorsal view. Coxa subcylindrical, distoventral part sparsely setose. Prefemoral part usually densely setose, with evident demarca-

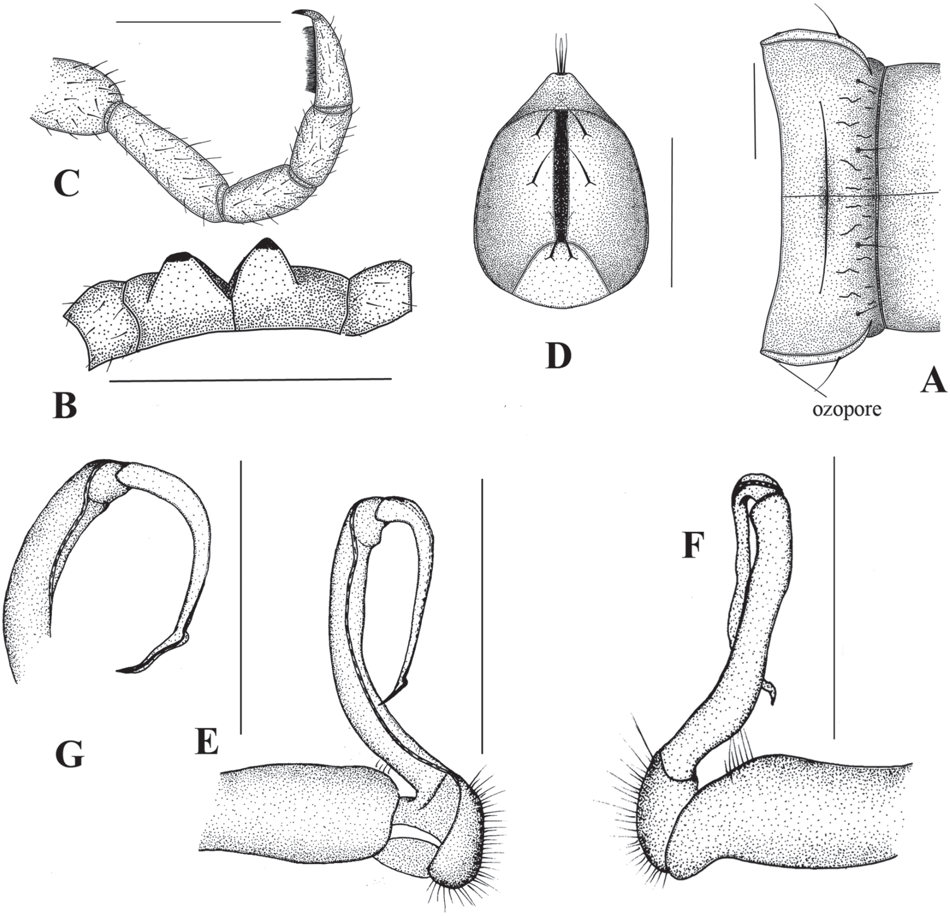


Figure 4. *Simplogonomorpha falcata* (Attems, 1953) comb. n. from Vietnam, BiDoup National Park **A** 10th body segment, dorsal view **B** sternal processes between 4th coxae, posteriovenral view **C** leg 10, lateral view **D** telson, ventral view **E–G** right gonopod **E** mesal **F** lateral, and **G** subdorsal view. Scale bar = 1 mm.

tion from both femorite and coxa. Femorite slender, much longer than coxite and a little curved down distally, separated from postfemoral part by an evident, subtransverse sulcus laterally and mesally. Solenomere simple, strongly curved down, slender, and tapering at tip. Tibiotarsus totally absent.

Prostatic groove runs mesally along femorite, distolaterad, and turns to lateral side, then running mesally, and ending at tip of solenomere.

Distribution. Vietnam, Lam Dong province, Bi Doup-Nui Ba National Park; Khanh Hoa province, Hon Ba Mountain; Laos PDR, Xieng Khoang

Remarks. New material does not much differ from Attems's description. Only minor difference is the presence of two separate cones between coxae 4 instead of only one small conal process in Attems's description. Recently, Chen et al. (2011) also published an illustration of gonopods of *Haplogonosoma falcatum* collected from the same locality, BiDoup National Park, Vietnam. Our material here fits well with their unevaluated illustration.

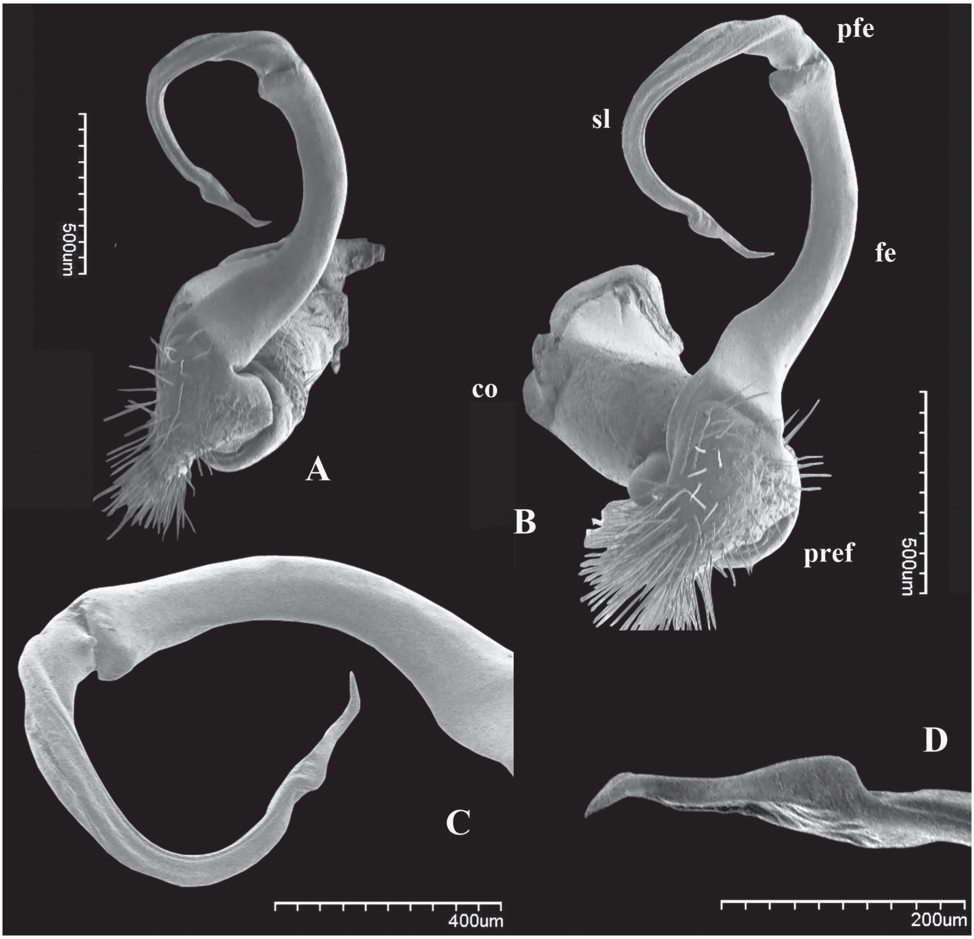


Figure 5. *Simplogonomorpha falcata (*Attems, 1953) comb. n. from Vietnam, BiDoup National Park, right gonopod: ventral **A** and mesoventral view **B, C** Tip of gonopod, ventral view **D**.

Key to representatives to all three genera (based on male characters)

1 Paraterga very weak, body looks almost cylindrical, colour uniformly light brown-yellowish. Gonofemorite very long and slender, without any modifications or processes. Postfemoral region consisting only a simple, strong, long solenomere. Solenophore totally absent (Figs 4–5)........ ***Simplogonomorpha***

– Paraterga well-developed with strong, triangular, posterio-lateral processes. Dorsal metazona colouration divided into dark brown anterior and much lighter posterior half, transversely separated by a deep sulcus....................... **2**

2 Gonofemorite large, slender and strongly concave, with a lamina on the mesal side. Postfemoral region with a long, large solenomere and a shorter solenophore, the latter basally with two processes, a laminar mesal (***lp***) and a more pointed lateral one (***pp***) (Fig. 3B)................................ ***Chamberlinius***

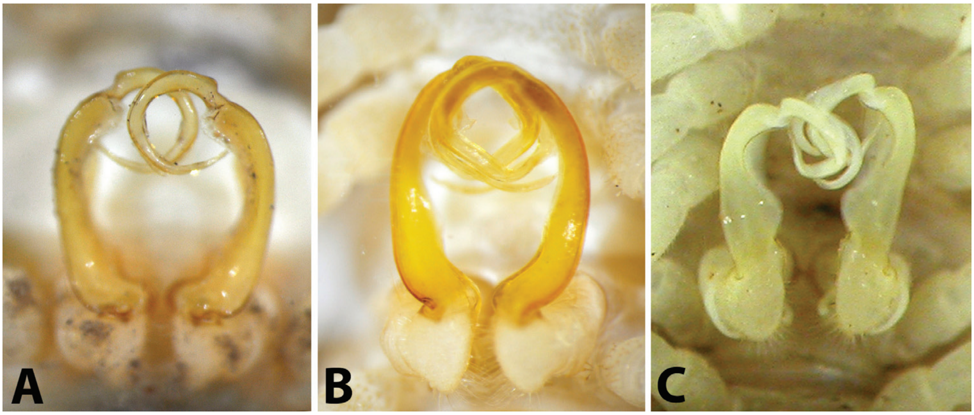


Figure 6. Male gonopods *in situ*, ventral view: **A** *Riukiupeltis jamashinai* (Miyako-jima Island) **B** *Chamberlinius hualienensis* (Okinawa-jima Island) **C** *Chamberlinius uenoi* comb. n. (Ishigaki-jima Island).

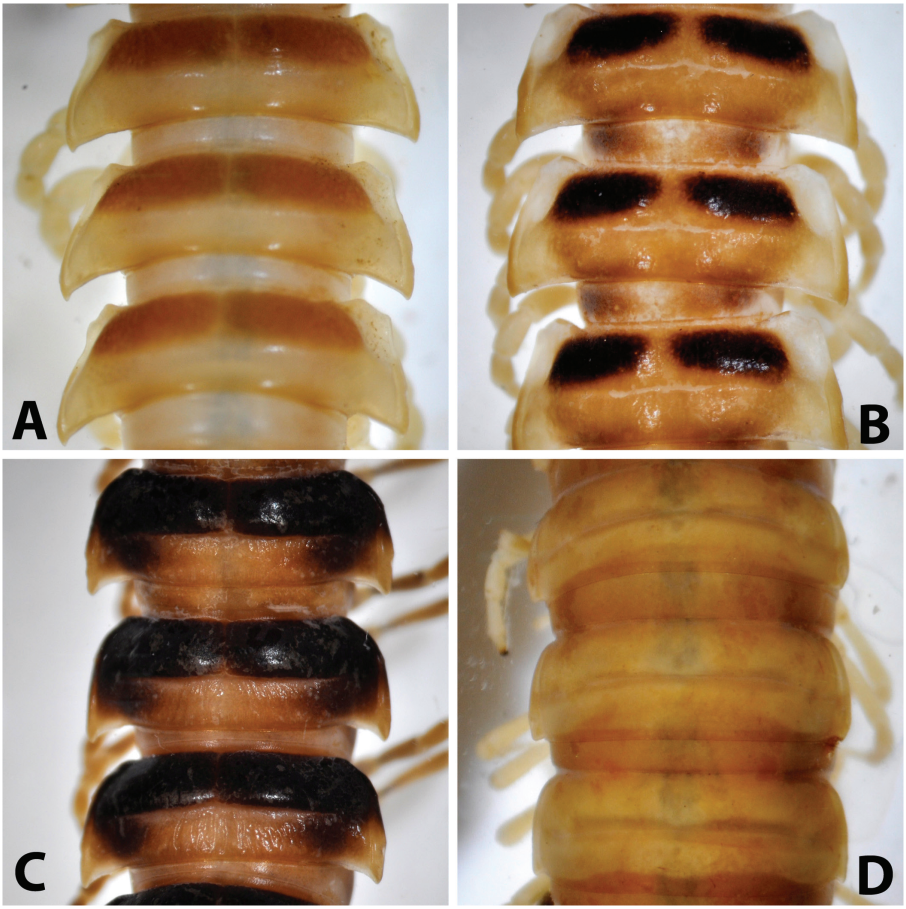


Figure 7. Midbody (11th-13th) segments, dorsal view: **A** *Riukiupeltis jamashinai* (Miyako-jima Island) **B** *Chamberlinius hualienensis* (Okinawa-jima Island) **C** *Chamberlinius uenoi* comb. n. (Ishigaki-jima Island) and **D** *Simplogonomorpha falcata* comb. n. (Vietnam, Hon Ba Mts.)

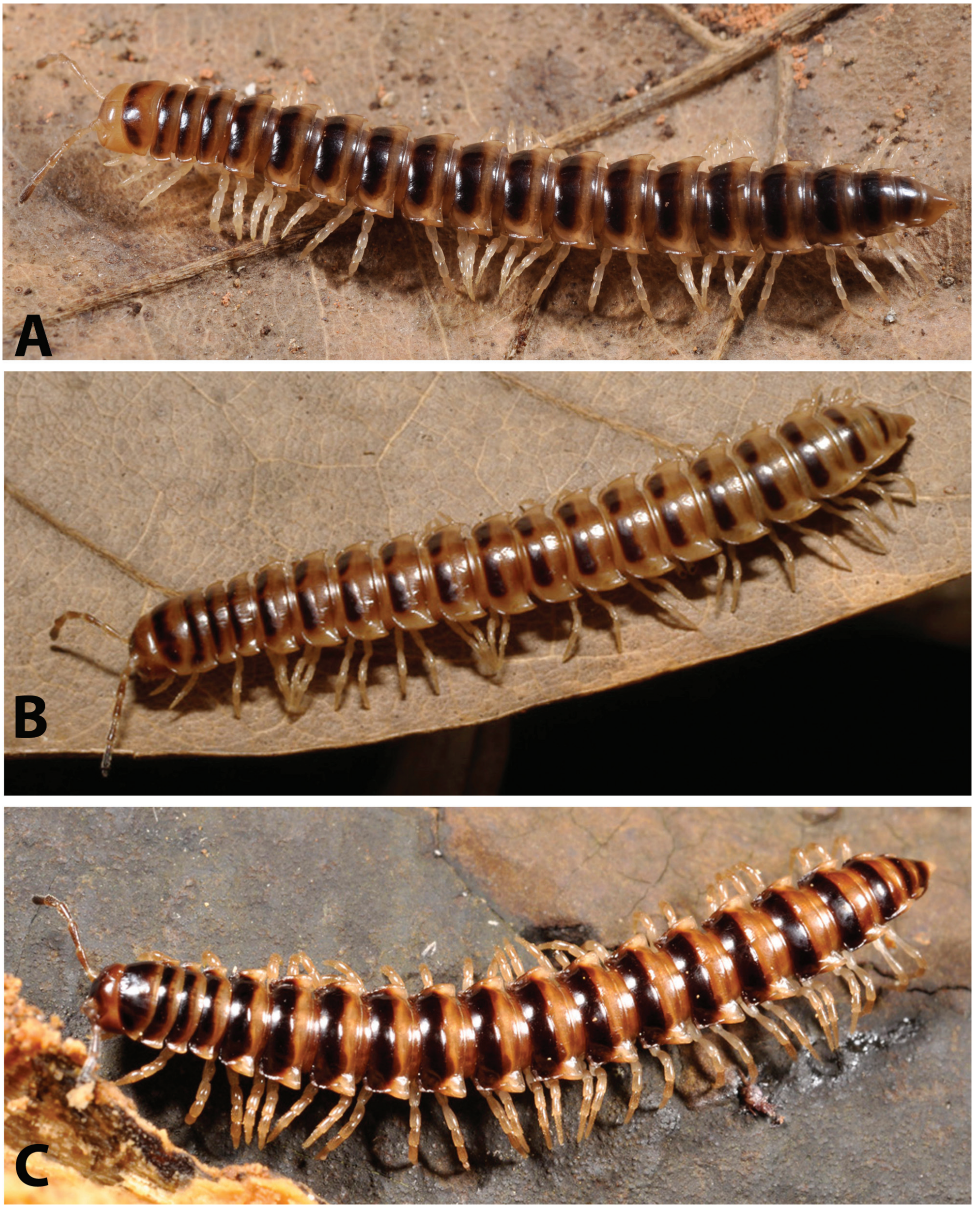
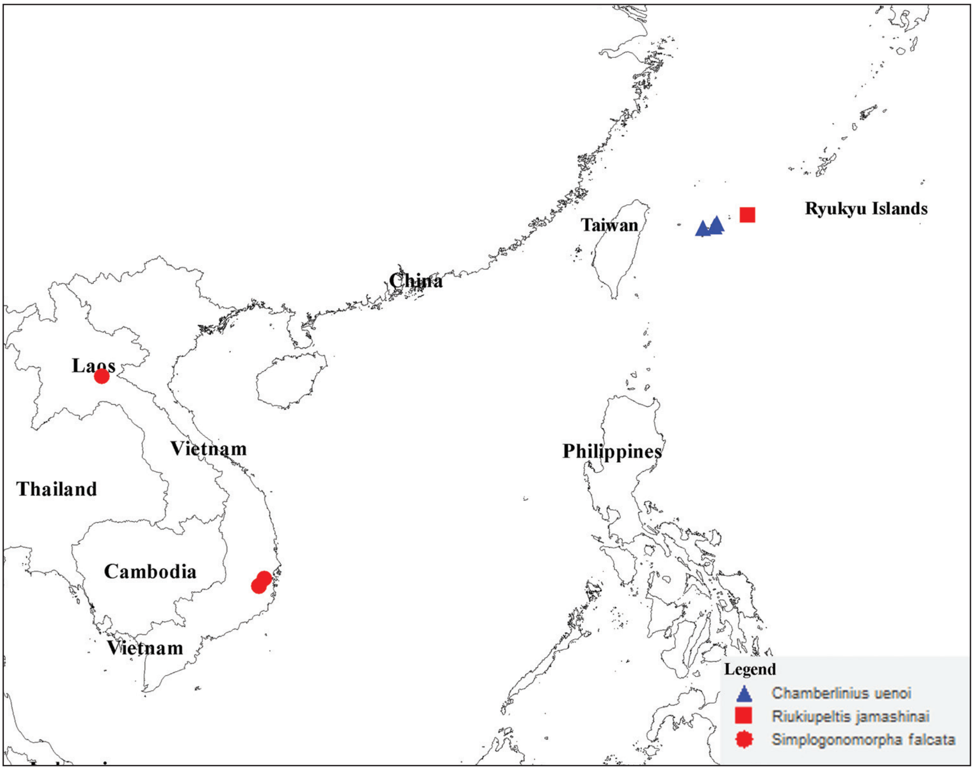


Figure 8. Habitus of millipedes: **A** *Riukiupeltis jamashinai* (live from Miyako-jima Island) **B** *Chamberlinius hualienensis* (live on Okinawa-jima Island) and **C** *Chamberlinius uenoi* comb. n. (live on Iriomote-jima Island).



Map 1. Distribution of three species *Chamberlinius uenoi* (Murakami, 1975) comb. n., *Riukiupeltis jamashinai* Verhoeff, 1939, and *Simplogonomorpha falcata* (Attems, 1953) comb. n.

– Gonofemorite only slightly curved, with a weak dorsal lamina, distal part swollen and membraneous. Postfemoral region consisting a thick, strong, free solenomere, and a short, somewhat hidden solenophore (Fig. 2) ....***Riukiupeltis***

In the key above, *Riukiupeltis* and *Simplogonomorpha* are represented by only one species each (*jamashinai* and *falcata*, respectively). *Chamberlinius*, on the other hand, includes at present five species: *Ch. hualienensis*, *Ch. piceofasciatus*, *Ch. pessior*, *Ch. sublaevus* (all keyed already by Chen et al. 2011), and *Ch. uenoi*, as added here.

Conclusion

As a result of our character comparisons, the taxonomic status of the following three species: *Riukiupeltis jamashinai* Verhoeff, 1939, *R. uenoi* Murakami, 1975 and *R. falcatus* (Attems, 1953) has been clarified. Based on significant morphological differences in body shape and gonopod structure, they all belong in three different genera. *Riukiupeltis jamashinai* having priority is maintained as such, whereas *uenoi* is transferred to *Chamberlinius* Wang, 1956, and a new genus, *Simplogonomorpha* gen. n., is erected to accommodate *falcatum*.

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RESEARCH ARTICLE

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Additional moults into 'elongatus' males in laboratory-reared *Polydesmus angustus* Latzel, 1884 (Diplopoda, Polydesmida, Polydesmidae) – implications for taxonomy

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Abstract

The number of stadia during post-embryonic development is supposed to be fixed in most species of the millipede order Polydesmida. For the first time since 1928, additional moults were observed in two males of *Polydesmus angustus* Latzel, 1884 reared in the laboratory. These 'elongatus' males sensu Verhoeff reached stadium IX instead of stadium VIII, with addition of a further podous ring (32 pairs of legs). One male had well-developed gonopods at stadium VIII, which regressed at stadium IX; the other had no gonopods at stadium VIII, which developed at stadium IX. The two cases correspond to the 'regressionis' and 'progressionis' forms described by Verhoeff in *Polydesmus complanatus* (Linnaeus, 1761), which confirms entirely his results. Additional moults appear to be associated with small body sizes and possible underlying mechanisms are discussed. Comparisons between millipede orders indicate that post-embryonic development is less strictly canalized in Polydesmida than in Chordeumatida. This implies that the adult number of body rings is of limited taxonomic value in Polydesmida and should not be viewed as a character of generic importance.

Keywords

post-embryonic development, anamorphosis, sexual maturity, stadium number variation, taxonomy

Introduction

In millipedes (Diplopoda), post-embryonic development occurs basically by anamorphosis, i.e. the number of body rings is small at birth (e.g., four podous rings bearing three pairs of legs, two apodous rings, plus the telson) and increases progressively at each moult. However, the relationships between developmental stages (= stadia) and sexual maturity vary greatly depending on the order (Enghoff et al. 1993). In most millipede orders, adults occur in several stadia within a species, either because the stadium at which maturity is reached varies among individuals, or because adults undergo further moults. In addition, adults in a given stadium often have a variable number of body rings (e.g. in Julida, Polyzoniida, Spirobolida, Spirostreptida). By contrast, development is much more canalized in the order Polydesmida, in which there is generally only one adult stadium in each species, with a fixed number of body rings (Mesibov 2011). In most polydesmidan species, adults are in stadium VIII, with the head, 18 podous rings, 1 apodous ring and the telson (in abbreviated form: 18+1+T); in other species, adults are in stadium VII (17+1+T); in a few species, the stadium and ring number of adults differ between the sexes, but with no intra-sex variation. In spite of obvious exceptions, e.g. in the genus *Devillea* Brölemann, 1902 (Xystodesmidae), in which adults probably occur in several stadia (Enghoff et al. 1993), the number of body rings in the adult is considered as fixed in most Polydesmida and is sometimes used in taxonomy (e.g. Djursvoll et al. 2000).

Surprisingly, Verhoeff (1916, 1928) reported the occurrence of an additional moult in a number of male and female *Polydesmus complanatus* (Linnaeus, 1761) (Polydesmidae) reared in the laboratory. Whereas adults of this species are normally in stadium VIII (18+1+T), some individuals were observed in stadium IX with a further podous ring (19+1+T). Verhoeff (1928) was convinced that the existence of these 'elongatus' specimens shed light on the evolution of ring numbers in millipedes and used it as an argument in his controversy with Brolemann (1921) over elongation vs. contraction. However, this additional moult in a polydesmid has sometimes been regarded sceptically, and Enghoff et al. (1993: p. 153) did not exclude the possibility of Verhoeff's mistakes in the counting of rings. More recently, intraspecific variability in the ring number of adult males was demonstrated in another polydesmidan millipede, the pyrgodesmid *Muyudesmus obliteratus* Kraus, 1960 (Adis et al. 2000).

Herein, the reality of the phenomenon observed by Verhoeff in *Polydesmus* is confirmed for the first time since 1928. During laboratory studies on another, closely related species, *Polydesmus angustus* Latzel, 1884, two cases of moult into stadium IX were observed in males. In the present paper, we first describe the conditions under which these moults occurred and some characteristics of the males before and after moulting, we briefly discuss possible underlying mechanisms, and then highlight implications of intraspecific variability in ring number for the taxonomy of Polydesmida.

Material and methods

Study species

The flat-backed millipede *P. angustus* is widespread in north-western Europe, west of the range of *P. complanatus* (Kime and Enghoff 2011). Its post-embryonic development consists of eight stadia, which can be determined by counting the number of body rings, and the sexes can be distinguished from stadium IV onwards (Enghoff et al. 1993). Towards the end of each immature stadium, millipedes build a chamber made of earthy faecal material, in which they coil during moulting. The last stadium is the adult (18+1+T), which dies after the breeding season. The life cycle is completed in either one or two years depending on the individuals. Under mean seasonal conditions, development time from egg hatch to adult emergence lasts about 10 months for annual individuals, plus a further 3 months in aestivation for biennial individuals (David et al. 1999).

Laboratory rearing

During experimental studies on the biology and ecology of *P. angustus*, hundreds of specimens were reared throughout their life cycle in the laboratory. Broods that were produced by adults from a field population living at Brunoy, 20 km south-east of Paris, were kept in lidded, transparent plastic boxes containing 1 cm of sieved soil and moist leaf litter. The boxes were placed in incubators fitted with a glass door and exposed to natural daylight. Temperature followed the mean monthly temperatures of the region of origin, with a daily thermoperiod of 4°C (David et al. 1999). The young from each brood were reared in groups up to stadia IV–V, sexed and then kept at a low population density to be monitored individually. Some individuals were fed on leaf litter alone throughout development, while others received a monthly pinch of dry yeast in addition to leaf litter, which greatly improves growth and female fecundity (David and Celerier 1997). Under those laboratory conditions, additional moults were observed in two males, one of which was reared with yeast and the other without.

Results

The first 'elongatus' male hatched in late August from eggs produced by field-collected parents (first generation in the laboratory). It was fed on leaf litter without dry yeast and emerged as a small adult (stadium VIII) in mid-September, at the age of 12 months. Its live weight was 15.3 mg, which is usually the weight of a stadium VII specimen in the field. The 8th leg-pair was transformed into apparently normal, well-developed gonopods. The male was left unmated and received a pinch of yeast in early October. It coiled into a moulting chamber on October 27th and then emerged as stadium IX (19+1+T) on November 18th. Its live weight was 22.4 mg. Although the examination of the exuvia confirmed that its gonopods were fully formed in stadium VIII, with all the characteristics of *P. angustus*, they were transformed regressively at stadium IX. The coxites were strongly developed, the coxal hooks clearly visible, but the telopodites were even more simplified and atrophied than in the figures shown by Verhoeff (1928: p. 691). This type of additional moult with regression of gonopods is similar to the 'regressionis' form described by Verhoeff (1916). This 'elongatus' male overwintered a second time and died in late March (Fig. 1).

The second male hatched in June from eggs produced by laboratory-reared parents (second generation in the laboratory). It was fed on leaf litter supplemented with yeast and emerged as stadium VIII on May 3rd, at the age of 10 months. It had the normal number of rings (18+1+T) but was extremely small. Its live weight was 9.2 mg, which is usually the weight of a stadium VI specimen in the field. It had no gonopods, the 8th leg-pair being replaced by small buds, as is usually the case in immature males of stadium VII. This male coiled into a moulting chamber in mid-May. Although it was accidentally frozen before emergence due to an incubator failure, the moulting process had already reached an advanced stage. An examination of the specimen dead before emergence clearly showed that it was about to give a male in stadium IX (19+1+T) with 32 leg-pairs and developed gonopods. This type of additional moult with appearance of gonopods is similar to the 'progressionis' form described by Verhoeff (1916).

Discussion

It is clear that the number of stadia is not completely fixed in millipede species of the family Polydesmidae. Additional moults described by Verhoeff (1916, 1928) in *P. complanatus* have been confirmed in adult males of *P. angustus*. In the latter species, it is a very rare phenomenon, which was observed in only two males and no females. Nevertheless, two modalities corresponding to Verhoeff's 'regressionis' and 'progressionis' forms have been confirmed, which lends considerable credibility to all the results reported by Verhoeff (1928). This also suggests that the additional moults briefly mentioned by Stephenson (1961) in a number of males of *Brachydesmus superus* Latzel, 1884 from the field, may not necessarily be due to confusion of species.

Possible underlying mechanisms

In this study, the two 'elongatus' males with 19 podous rings were obtained under controlled laboratory conditions and did not experience environmental stress in terms of temperature, humidity and photoperiod. However, an obvious difference between these males and normally developing animals was their small body size at stadium VIII. Simi-

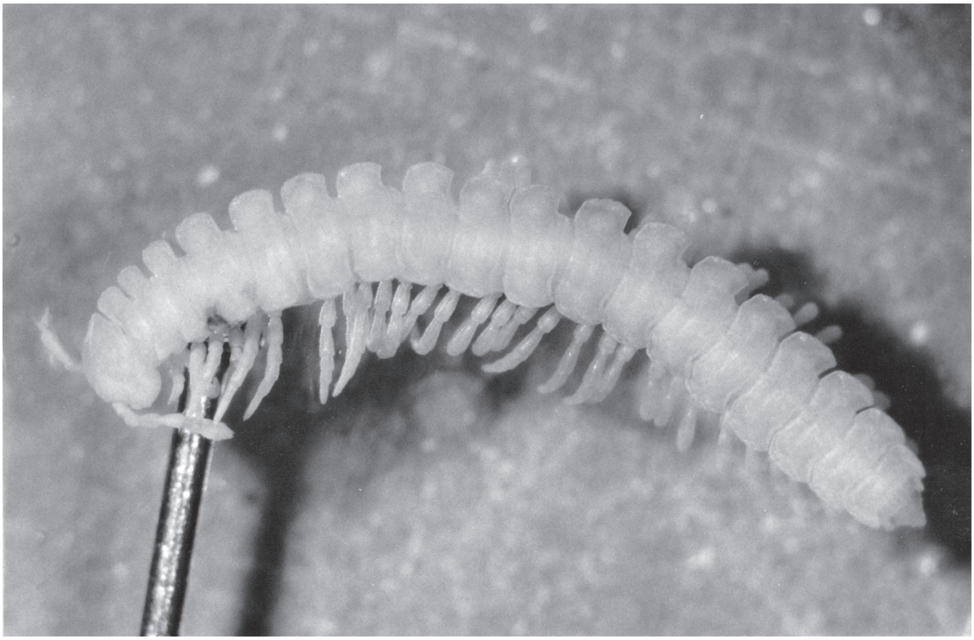

Figure 1. Dorsal view of a *Polydesmus angustus* male of stadium IX after its death at the age of 19 months. The trunk consists of 19 podous rings – bearing 32 pairs of legs plus regressed gonopods (8th leg-pair) – and one apodous ring anterior to the telson.

larly, the first 'elongatus' specimens obtained by Verhoeff (1916) were from a poorly-fed brood that yielded very small adults (the so-called 'forma nana'), and most of those he obtained later were less than 20 mm long at stadium VIII (Verhoeff 1928). Body size may thus be critical for the appearance of 'elongatus' specimens. A small size can result from poor quality food (David and Celerier 1997), although this is not a valid explanation for the smaller 'elongatus' male obtained in *P. angustus*, which was reared on leaf litter supplemented with yeast. In an experiment with a species of the order Spirobolida in which the mode of anamorphosis is very different from that of Polydesmida, Berns and Keeton (1968) showed that semi-starvation resulted in smaller juveniles, and these underwent a greater number of moults before maturity than well-fed individuals.

Hormone imbalance is undoubtedly involved in the occurrence of additional moults, and body size may play a role in this respect. Although little is known about the hormonal control of development in millipedes (Descamps et al. 1990; Hopkin and Read 1992), hypotheses based on knowledge from other arthropod classes can be put forward. In insect species with variable numbers of instars, such as the Lepidoptera *Manduca sexta* (Linnaeus, 1763) (Sphingidae) and *Malacosoma disstria* Hübner, 1820 (Lasiocampidae) (Kingsolver 2007; Etile and Despland 2008), further larval moults occur only in small individuals, because attainment of a critical weight is the signal that stops juvenile hormone production (Davidowitz et al. 2003). A similar mechanism can be hypothesized for the additional moult in the 'progressionis' form of *Polydesmus*, which is typically the occurrence of a further immature stadium during development.

The 'regressionis' form in males is more difficult to interpret. In this case, small males reach stadium VIII with the normal number of rings and well-developed gonopods. However, according to Verhoeff (1928), these males are unable to breed because other adult characters are missing, especially the male openings on the coxae of the second pair of legs. These characters appear in stadium IX, while the gonopods are transformed regressively. A mixture of development and regression of secondary sexual characters during the additional moult is difficult to interpret in terms of hormonal control.

It is clear, however, that the additional moult with regression of gonopods in *Polydesmus* is not akin to periodomorphosis (Verhoeff 1923). This phenomenon, which is well known in the order Julida, involves the appearance of intercalary males with regressed sexual characters between two adult stadia (Enghoff et al. 1993). If *Polydesmus* males in stadium VIII are not sexually mature despite their well-developed gonopods, their moult to stadium IX is by no means the first stage in periodomorphosis (Verhoeff 1928; Sahli 1968). While some secondary sexual characters regress, others develop in stadium IX, so that 'regressionis' males are quite different from true intercalary males. Moreover, the next stage in periodomorphis, i.e. a further moult into mature males of stadium X, has never been observed in *Polydesmus*.

Taxonomic implications

The confirmation that additional moults can occur in Polydesmida, the post-embryonic development of which is generally assumed to consist of a fixed number of stadia, has implications for taxonomy. In *Polydesmus*, adults with a further body ring are quite capable of surviving and Verhoeff (1928) even showed that 'elongatus' females of *P. complanatus* were able to breed. Although such individuals have not been reported so far in field populations of *Polydesmus*, they might be encountered besides normally developing adults under specific ecological conditions, especially in populations composed of small individuals. Clearly, it is biologically possible. Therefore polydesmidan millipedes that have very similar characteristics, particularly the male gonopods, but different numbers of rings in the adult, might belong to the same species. They should not be automatically classified as distinct genera. For example, Demange (1970) stressed that the polydesmids *Brachydesmus proximus* Latzel, 1889 (17+1+T) and *Polydesmus geochromus* Attems, 1952 (18+1+T) have virtually identical gonopods; the paradoxosomatids *Paradoxosoma granulatum* Daday, 1889 (17+1+T) and *Trachydesmus simonii* Daday, 1889 (18+1+T) also have virtually identical gonopods. Distinctions at the generic level for such closely related organisms were criticized (Jeekel 1968; Demange 1970), and the latter author correctly argued that these differences in ring number could reflect environmentally induced variation within a single species. In the recent scientific literature, Shelley (2000) chose the option to classify adult males with 17 and 18 podous rings in the same sphaeriodesmid species, *Desmonus pudicus* (Bollman, 1888). At most, such differences could reflect speciation, assuming that each ring number has already been fixed in reproductively isolated populations. But the number of rings as such should no longer be viewed as a character of generic importance in Polydesmida. Its use in phylogenetic analyses, working on the assumption that this character shows no intraspecific variation (Djursvoll et al. 2000), perpetuates divisions which may be artificial, such as the genera *Brachydesmus* (17+1+T) and *Polydesmus* (18+1+T).

Conclusion

Enghoff et al. (1993) coined the term teloanamorphosis to describe a mode of anamorphosis in which the number of moults and the number of rings added at each moult are fixed within each species (or each sex of a given species). In millipedes, this type of post-embryonic development is characteristic of the orders Chordeumatida and Polydesmida, with some interspecific variation in the number of stadia to maturity. However, there appears to be a difference between the two orders. Whereas no additional moults have ever been mentioned in the Chordeumatida, the data available to date show that there are various degrees of intraspecific variability for the stadium number in Polydesmida, at least in *Devillea*, *Muyudesmus* and *Polydesmus*, and possibly in other genera mentioned above. The number of body rings in the adult appears therefore to be of more limited taxonomic value in this order.

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Detecting taxonomic signal in an under-utilised character system: geometric morphometrics of the forcipular coxae of Scutigeromorpha (Chilopoda)

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Abstract

To date, the forcipules have played almost no role in determining the systematics of scutigeromorph centipedes though in his 1974 review of taxonomic characters Markus Würmli suggested some potentially informative variation might be found in these structures. Geometric morphometric analyses were used to evaluate Würmli's suggestion, specifically to determine whether the shape of the forcipular coxa contains information useful for diagnosing species. The geometry of the coxae of eight species from the genera *Sphendononema*, *Scutigera*, *Dendrothereua*, *Thereuonema*, *Thereuopoda*, *Thereuopodina*, *Allothereua* and *Parascutigera* was characterised using a combination of landmark- and semi-landmark-based sampling methods to summarize group-specific morphological variation. Canonical variates analysis of shape data characterizing the forcipular coxae indicates that these structures differ significantly between taxa at various systematic levels. Models calculated for the canonical variates space facilitate identification of the main shape differences between genera, including overall length/width, curvature of the external coxal margin, and the extent to which the coxofemoral condyle projects laterally. Jackknifed discriminant function analysis demonstrates that forcipular coxal training-set specimens were assigned to correct species in 61% of cases on average, the most accurate assignments being those of *Parascutigera* (*P. guttata*) and *Thereuonema* (*T. microstoma*). The geographically widespread species *Thereuopoda longicornis, Sphendononema guildingii*, *Scutigera coleoptrata*, and *Dendrothereua linceci* exhibit the least diagnostic coxae in our dataset. *Thereuopoda longicornis* populations sampled from different parts of East and Southeast Asia were significantly discriminated from each other, suggesting that, in this case, extensive synonymy may be obscuring diagnosable inter-species coxal shape differences.

Keywords
Centipedes, forcipules, eigenshape, Procrustes superposition, canonical variates analysis

Introduction

The Scutigeromorpha (Chilopoda) is the only extant representative of the centipede subclass Notostigmophora. These centipedes retain several primitive characters such as compound eyes, a domed head capsule, and deposition of the spermatophore on the ground rather than on a web. These characteristics, together with molecular sequence data, identify them as the sister group of all other centipedes (Murienne et al. 2010).

Scutigeromorph taxonomy in its present form was largely established by K. W. Verhoeff in a series of studies that spanned the first half of the 20th century. Verhoeff (1905) was also the first investigator to present a hypothesis of phylogenetic relationships for the group. He named most of the genera (Verhoeff 1904, 1905, 1925, 1944) and a large number of species, many of which have been synonymised subsequently (see Würmli 1973a,b, 1977, 1978, 1979, 2005). Despite the inclusion of new morphological character data (e.g., from scanning electron microscopy) and extensive molecular sequencing (Edgecombe and Giribet 2006, 2009), the taxonomy and phylogenetic relationships of the ca 100 valid scutigeromorph species remain controversial, in part because many aspects of this group's morphology are highly conserved.

Among such complex, but apparently conservative, character systems are the forcipules, the appendages of the first trunk segment that are a functional part of the head and house the poison gland. Scutigeromorph forcipular coxae are separated from a vestigial sternite (Manton 1965) and each coxa bears four long spine-bristles along its anterior margin (Fig. 1). Würmli (1974) drew attention to the importance of the shape – the relative lengths and widths of the coxa — and the prominences on the inner margin of these structures. However, the degree to which these shape characters can be used to identify taxa (either species or infra/supraspecific groups) reliably has never been subjected to systematic investigation. Indeed, the forcipules have played almost no role in scutigeromorph systematics to date.

Geometric morphometrics has been used for the past 25 years to quantify biological form via the use of landmark and semi-landmark data (Rohlf and Marcus 1993, Adams et al. 2004, MacLeod 2002a, 2002b, 2005; see also Bolton et al. 2009 for an application to the female gonopods of Scutigeromorpha). Whereas a number of authors have suggested that morphometrics could be useful in resolving traditional taxonomic characters and contributing to taxonomic and phylogenetic analysis (e.g., MacLeod 2002a), morphometric approaches have traditionally been used to evaluate characters that have been recognized by taxonomists via qualitative inspection. In the case of the scutigeromorph forcipules, despite their morphological complexity these structures have defeated qualitative analysis; taxonomically and phylogenetically in-

Figure 1. Ventral view of head and forcipules of *Thereuopoda longicornis* placed in a standard horizontal position. BM 1952.9.8.574-575, Kuching, Sarawak, Malaysia.

formative morphological characters have necessarily been sourced from other parts of the body.

The primary objective of this investigation was to determine the degree which the forcipular coxae can be used to characterise scutigeromorph taxonomic and phylogenetic groups accurately based on an assessment of their shape. In doing so, we also explored the extent to which Procrustes principal component analysis (see MacLeod 2010) as well as canonical variates analysis (CVA) and shape models calculated for the CVA ordination space can help identify characters that add support for existing or alternative taxonomic placements.

Methods

Taxonomic sampling

Specimens fixed in ethanol were sourced from The Natural History Museum (London). Specimens of the three Australian species were sourced from the Australian Museum (Sydney) and the Queensland Museum (Brisbane); specimens of two Dominican Republic species were located in the collections of the U.S. National Museum of Natural History.

Landmark and outline data were collected for 108 specimens (Table 1), representing eight species and eight genera within two of the three scutigeromorph families. The Neotropical/Afrotropical family Pselliodidae is here represented by *Sphendononema guildingii* (Newport, 1844), and the family Scutigeridae, where sampling was densest, includes members of both recognized subfamilies, Scutigerinae and Thereuoneminae. The Scutigerinae were represented by *Scutigera coleoptrata* (Linnaeus, 1758) and *Dendrothereua linceci* (Wood, 1867); the Thereuoneminae by *Thereuonema microstoma* (Meinert, 1886), *Thereuopoda longicornis* (Fabricius, 1793), *Thereuopodina queenslandica* Verhoeff, 1925, *Allothereua maculata* (Newport, 1844), and *Parascutigera guttata* Verhoeff, 1904. All species were chosen on the basis of being members of accessible collections with sample size adequate for statistical analysis, and to represent a broad sample of generic/subfamilial diversity. The remaining scutigeromorph family, Scutigerinidae, was not included as too few specimens were available. Voucher details for all specimens used in this study are listed in the Appendix (Table 1 therein).

The species concept for *Dendrothereua linceci* follows Würmli (1973b), applying this name to populations distributed from the southern U.S. to Panamá. Analyses of molecular data suggest that multiple species may be represented in this aggregation (Edgecombe and Giribet 2009), but using the traditional concept of a widespread species allows exploring the variability in this taxonomic grouping. Identifications of other widespread species follow their most recent revisions (*Thereuonema microstoma* = *T. syriaca*: Würmli 1975; Stoev and Geoffroy 2004; *Scutigera coleoptrata*: Würmli 1977; *Sphendononema guildingii*: Würmli 1978; *Thereuopoda longicornis*: Würmli 1979; *Parascutigera guttata*: Edgecombe and Giribet 2009). As in the study of Bolton et al. (2009), the name *Allothereua maculata* is applied to populations from arid parts of New South Wales and South Australia that belong to *A. maculata* (sensu Verhoeff 1925), though their conspecificity with the Western Australian type material is dubious.

Specimens were chosen so that at least one of the forcipular coxae and the spine bristles on its anterior margin were visible and complete. To minimise the effects of

Table 1. Sample number and distribution of species employed in the present study

Species	**Sample number (N)**	**Distribution**
Sphendononema guildingii	5	Central and South America
Scutigera coleoptrata	12	Mediterranean, cosmopolitan by introduction
Dendrothereua linceci	13	North-Central America, Caribbean
Thereuonema microstoma	14	East Africa, Middle East
Thereuopoda longicornis	19	India, southeast Asia
Thereuopodina queenslandica	14	Northeastern Australia
Allothereua maculata	13	Southern Australia
Parascutigera guttata	18	Northeastern Australia
Total	**108**	

variation due to potential shape changes in ontogeny, specimens are all mature or nearly mature (maturus and pseudomaturus stages of Verhoeff 1904) apart from some of the smaller *Dendrothereua linceci*.

Image capture and landmarks

In order to quantify the outline of each forcipular coxa, specimens were placed ventral surface uppermost and pinned to a horizontal standard orientation (i.e., the forcipular coxae positioned as horizontal as possible) (Fig. 1). Each specimen was imaged perpendicularly using a Leica MZ16 stereomicroscope with a Zeiss Axiocam™ high-resolution imaging system.

Imaging the specimens in the correct orientation while in alcohol proved difficult as many specimens were not perfectly straight and even the slightest movement disturbed the alcohol and blurred the image. Additionally, the forcipular coxae are not completely flat, rendering some areas of the coxa out of focus. To overcome focusing problems, a stack of images of each specimen at different focal depths was taken and the set then merged to form a single, extended focus composite using HELICON FOCUS™ (Helicon Software Ltd.) software. All composite images were then cropped, placed on a black background, and contrast adjusted using Adobe PHOTOSHOP™ software while referring to the original images for guidance. Images were excluded if (1) the original image stack was insufficiently focused to accurately detect the coxal outline and/or the base of all the spine-bristles, and/or (2) their orientation did not conform to an acceptable standard.

Media Cybernetics' IMAGE-PRO PLUS™ software was used to collect landmarks and outline co-ordinate points from the right and the left coxae individually. Symmetry between the right and left coxae was established (see Results section), and subsequently the left coxal landmark and outline coordinate points were reflected across the y-axis, only one coxa being used per specimen to eliminate redundancy. Ten landmarks (Fig. 2) were manually located from the interior to the exterior part of each coxa. The first landmark (L1) was calculated by drawing the longest diagonal line from the anterolateral to the posteromedial edge of the coxa, the second landmark (L2) was placed at the coxofemoral condyle, and landmarks L3-L10 were situated at the base of the projection bearing each spine-bristle. In addition, 100 equally-spaced outline semi-landmark co-ordinate points were obtained by automatic tracing along the mesial, posterior and lateral edges of the image from landmarks L10 to L3. The outline along the anterior margin (from landmarks 3 to 10) was obtained manually as the spine-bristles were not used in the shape analysis. These structures are very fragile and were disarticulated in a large subset of the specimens available or their length was partially obscured by other structures. Accordingly the manual tracing truncated the spine-bristles across their level of insertion into the coxae.

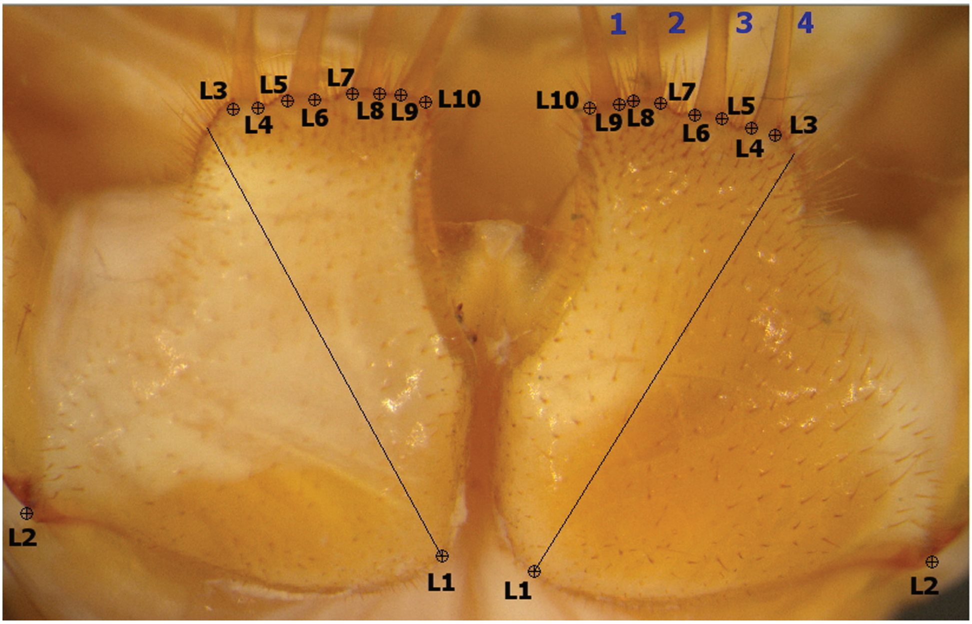

Figure 2. Landmarks (L1-L10) used in morphometric analysis. Diagonal line to L1 is the longest line from anterolateral to posteromedial corners of the coxa. Spine-bristles numbered 1-4 (blue) from interior to exterior. Throughout text, left and right coxae refer to dorsal orientation (inverted 180° relative to this ventral view).

Shape

Initially, combinations of both landmark and outline data were analysed using routines written in Wolfram Research's MATHEMATICA™ software using the semi-landmark sampling protocol described by MacLeod (1999). This protocol, part of the extended eigenshape analysis procedure, combines the coordinates of semi-landmark points along the outline and landmarks placed at comparable geometrical points which constrain the sequencing of the boundary coordinate points by forcing them into alignment, thereby reducing the degree of shape variation generated through biological miscorrespondence (MacLeod 1999). For this investigation a shape accuracy tolerance criterion of 0.975 was used to control the outline interpolation process. The analysis generated a series of *x-y* boundary outline (= semi-landmark) coordinate values that were used to represent the outline shape of each coxa.

No attempt was made to 'slide' the resulting semi-landmark points to positions of minimum bending energy relative to the sample mean as has been advocated for use in the analysis of semi-landmark data by some (e.g., Bookstein 1996, Zelditch et al. 2004). Such a transformation would destroy the shape correspondences that are the point of morphometric analysis. Moreover, all current implementations of the sliding semi-landmark approach of which we are aware do not slide the semi-landmark along the boundary outline itself, but rather along straight lines tangent to the boundary outline (see Rohlf's documentation for the tpsRelw program, available as part of that program at the SB Morphometrics web site: http://life.bio.sunysb.edu/morph/). This convention has been adopted to simplify the computations required to reposition the semi-landmarks. However, use of this approach to semi-landmark adjustment obviously does not achieve a configuration of minimum bending energy for the curve that was measured because the act of sliding semi-landmarks along local tangents deforms the original measured curve. This wholly artificial deformation will not apply uniformly to the entire shape, but will be more pronounced in regions that exhibit high outline curvatures. Irrespective of this consideration, experiments have shown that minimal semi-landmark sliding results from the high-density sampling program used in extended eigenshape analysis.

The output of semilandmark interpolation procedure was aligned using Procrustes (GLS) superposition, which minimizes differences in position, scale, and rotation for sets of landmarks and semilandmark points (MacLeod 2009a). The superimposed coordinates were then analysed via PCA using the covariance matrix as a basis for the assessment of shape similarity. This technique explores the relations between variables to create new independent variables, the set of principal components, which represent a variance-optimised and mutually independent set of shape descriptors derived from the information contained in the original measurement set, reducing the dimensionality of the data set (Dunteman 1989; MacLeod 2005). In addition, this set of variables (= eigenvectors) can be used to define an ordination space that can be used to graphically portray shape relations and to create models of shape deformation trends that graphically embody the geometric meanings of the variables (Bookstein 1991; MacLeod 2009b, 2010).

Subsequently, canonical variates analysis (CVA) was performed on the PCA scores on a shape variance-optimised subset of the PCA axes to maximize the ratio of between-group and within-group variation for the eight species, thus discriminating between the groups (Zelditch et al. 2004). CVA was also used to investigate asymmetry differences between the right and left coxae, sexual dimorphism, and whether these shapes are informative for geographical patterns at an infraspecific level.

The geometric interpretation of the CVA axes that support group separation was then assessed using a CVA modelling procedure that projects points from the CVA space into the original PCA variable space to calculate a series of shape models that express the major shape trends involved in inter-group separation (see MacLeod 2007; Bolton et al. 2009).

Results

In order to determine whether left and right coxae exhibit shape differences, data collected from both sides of specimens were subjected to CVA after coordinate data from the right coxae were reflected across the *y*-axis. No obvious differences in the scatter of left and right coxa along the first discriminant axes were evident. This result was then confirmed statistically using a likelihood ratio test (see Manley 1994; $\phi = 9.66$, df = 12, $p > 0.001$). This same procedure was used to test for significant male-female shape differences ($\phi = 41.11$, df = 24, $p > 0.001$). In both cases the null hypotheses of no between group shape differences could not be rejected. Additionally, a measurement error analysis was carried out using two randomly selected replicates of 12 specimens to estimate the level of data-collection accuracy that was achieved for the data set as a whole. This analysis also identified no significant statistical coxal shape differences ($\phi = 0.385$, df = 9, $p > 0.001$) between replicate data collection sessions.

Ordinations of coxal outline shape projected along the first few Procrustes PCA axes for each species analysed independently did not reflect any obvious infraspecific clusters. The structure of infraspecific coxal shape was further investigated by grouping the data within *Thereuopoda longicornis* and *Dendrothereua linceci* by geographic locality. These geographically widespread species were selected because sufficient specimens were available from enough localities to allow a more rigorous comparison. Results of a CVA of the Procrustes PCA score data rejected the null hypothesis of no significant infraspecific differences in coxal shape between geographic groups in *Thereuopoda longicornis* (likelihood ratio: $\phi = 71.14$, df = 22, $p < 0.001$). This result was supported by unexpectedly impressive discriminations by the CVA results. The set of CVA discriminant function axes revealed that specimens of *T. longicornis* were assigned to their correct geographic group with 89% accuracy (Table 2), indicating marked and consistent infraspecific coxal shape differences. Furthermore, the inspection of CVA scatterplots (Fig. 3) shows that specimens from each of six pre-defined geographic regions within the species' ordinations plot close to each other in the discriminant space. Results for *Dendrothereua linceci* are shown in the Appendix (Fig. 1).

When the sample of all eight species was pooled, Procrustes PCA identified a total of 12 axes that were required to account for 95% of the observed coxal shape variation (Fig. 4). Subsequent CVA analysis of the Procrustes PCA scores for these

Table 2. CV discriminant function analysis of *Thereuopoda longicornis* geographic data.

Groups	**Sumatra**	**N. Borneo**	**Peninsular Malaysia**	**Thailand**	**Burma**	**China**	**Total Correct**	**% Correct**
Sumatra	1	0	0	0	0	0	1	100
N. Borneo (Sarawak)	0	2	0	0	1	0	2	67
Peninsular Malaysia	0	1	5	0	0	0	5	83
Thailand	0	0	0	2	0	0	2	100
Burma	0	0	0	0	6	0	6	100
China	0	0	0	0	0	1	1	100
						Total Correct	**17**	**89**

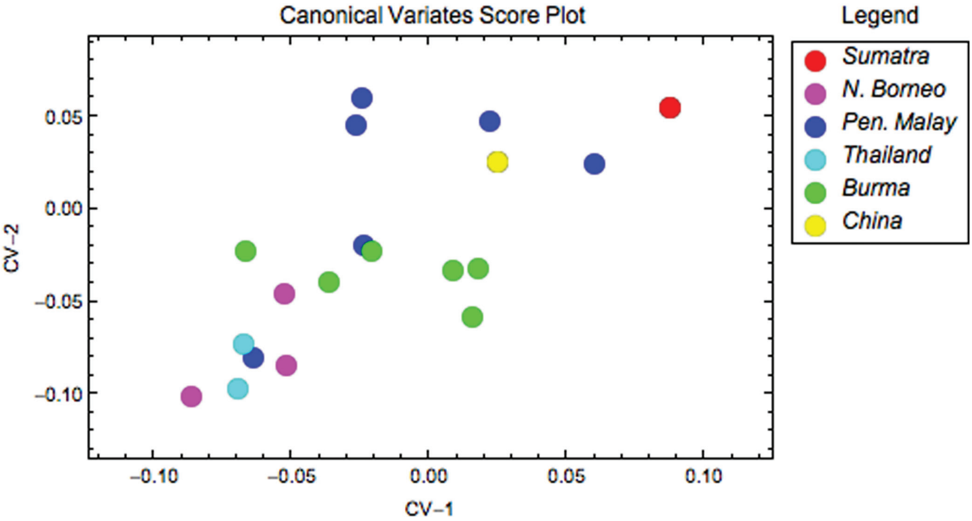

Figure 3. *Thereuopoda longicornis* scatterplot of coxal shape data along the discriminant subspace formed by the first two CV axes, which together account for 74.17% of observed between-group shape variation.

12 latent shape variables revealed a clear tendency to group separation that is more pronounced for some species (*Thereuopodina queenslandica*, *Parascutigera guttata*, *Thereuonema microstoma* and *Allothereua maculata*) than for others (*Thereuopoda longicornis* and *Sphendononema guildingii*; see Fig. 5). There is a high degree of overlap evident in plots of species scatter in low-dimensional CVA subspaces. However, the degree of this true overlap among species point clouds is exaggerated in such plots as only two or three (out of the seven) discriminant axes were used in constructing the figure. Using the complete set of CV discrimination function axes, the overall proportion of correct specimen assignments (77%) was unexpectedly high for a character complex previously regarded as being of little taxonomic value. This, along with the likelihood ratio test results ($\phi = 308.5$, df = 84, $p < 0.001$), indicates the presence of substantial and consistent between-species coxal shape differences for these data. *Parascutigera guttata*, *Allothereua maculata* and *Thereuonema microstoma* exhibit the most distinct coxal shape with 94%, 85%, and 79%, respectively, of specimens correctly assigned based on discriminant function analysis, whereas the least distinct were *Sphendononema guildingii* (60%) and *Thereuopoda longicornis* (68%). A jackknifed identification test to assess the stability of the discriminant axes (Table 4) indicates that, across the dataset as a whole, the individual training set outlines were assigned to the correct species with 61% accuracy. The most stable results, with reference to generalized group identification, are those for *Parascutigera guttata* and *Thereuonema microstoma* (89% and 71% correct identifications, respectively).

Figure 6 illustrates the forcipular coxal models that project three of seven CVA axes back into the space of the original Procrustes PCA axes. These three axes express more than 75% of between-species shape differences. The major shape trends illustrated in

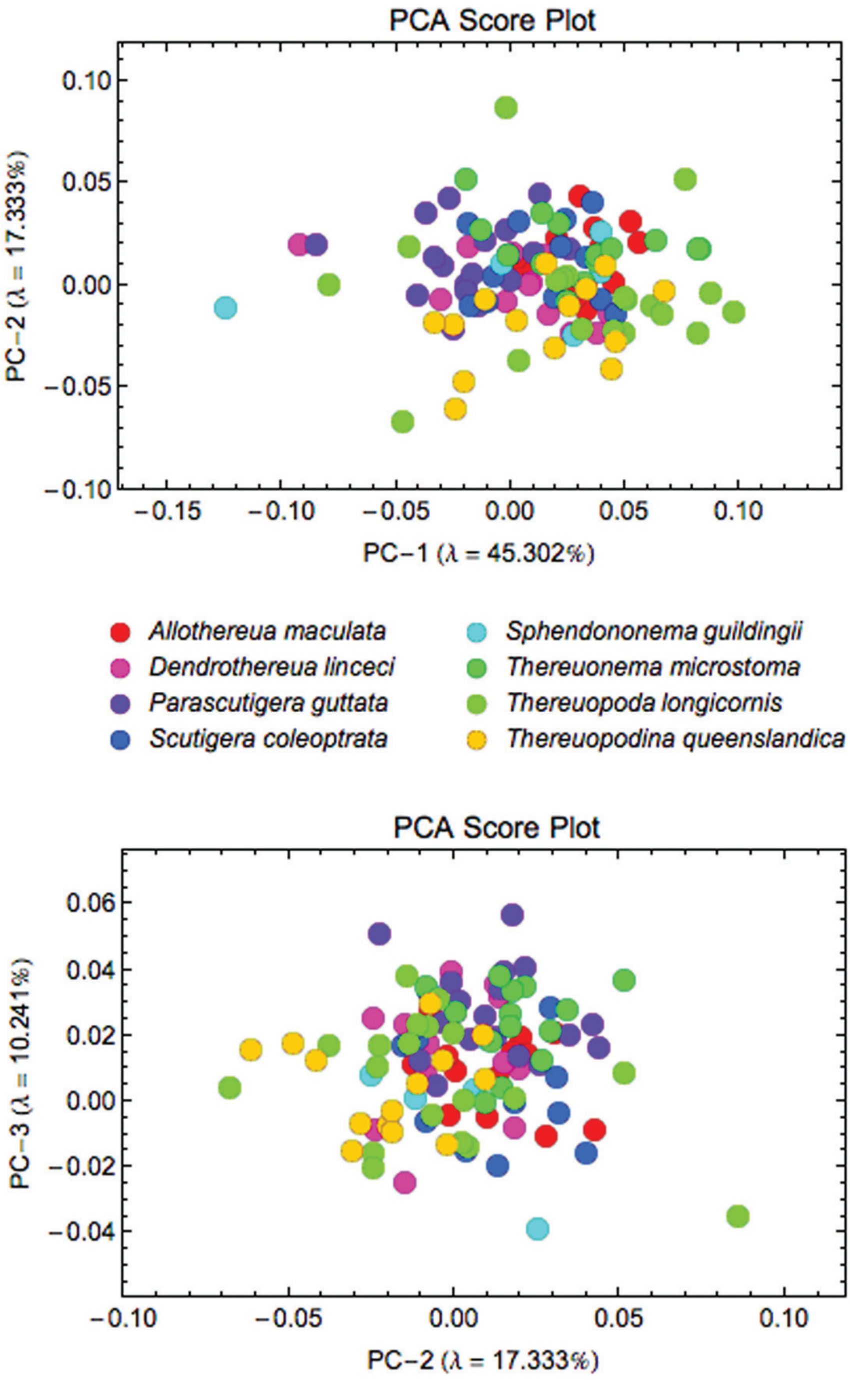

Figure 4. Scatterplots of Procrustes PCA scores for coxal shape data. The first two shape variation axes (top) together account for 62.63% of the observed shape variation; PC-2 and PC-3 axes (bottom) together account for 27.58% of the observed shape variation.

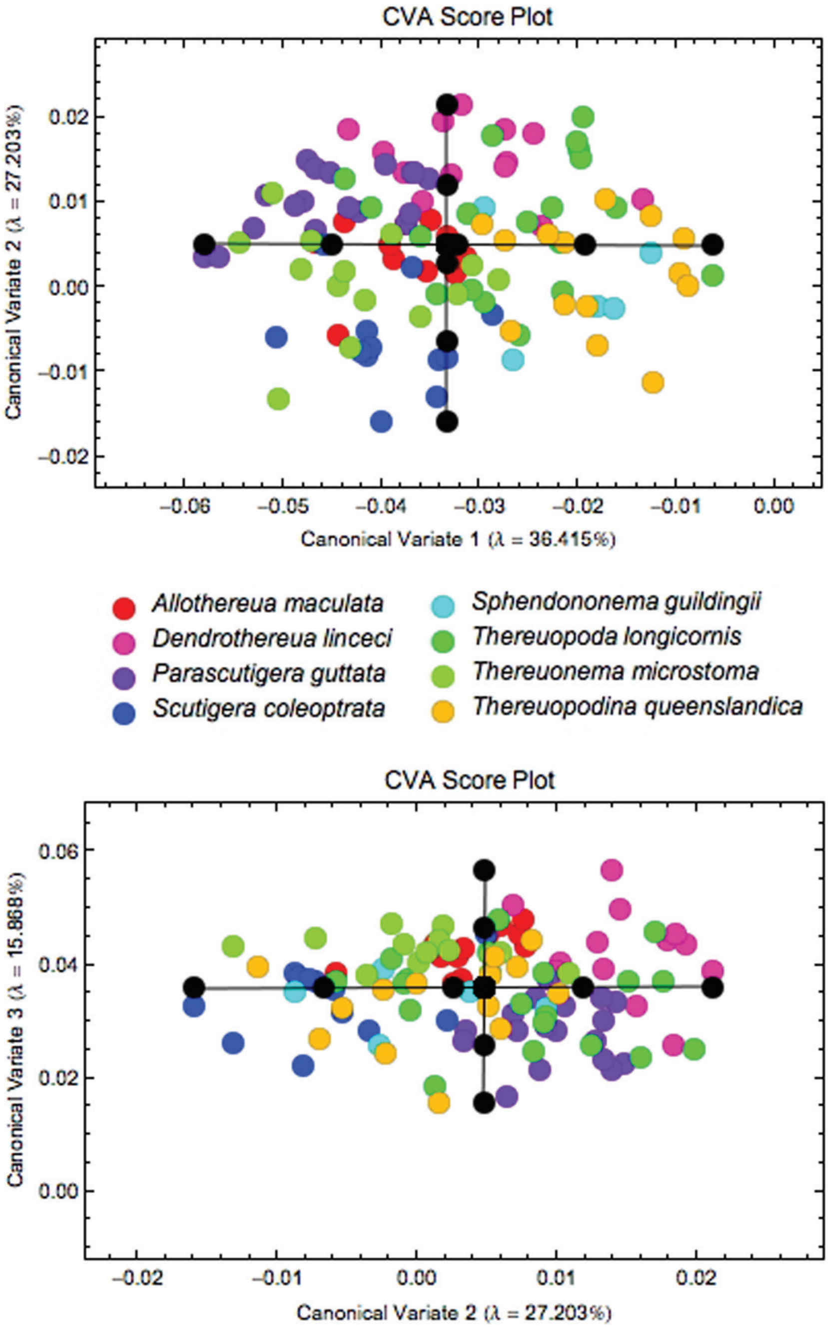

Figure 5. Results of the CVA of coxal shape data for all eight species, showing the subspaces formed by the first three discriminant axes, which together account for more than 79% of observed between-group shape variation. Within each subspace plot the black circles represent the coordinate locations for each of the five along-axis shape models depicted in Fig. 6.

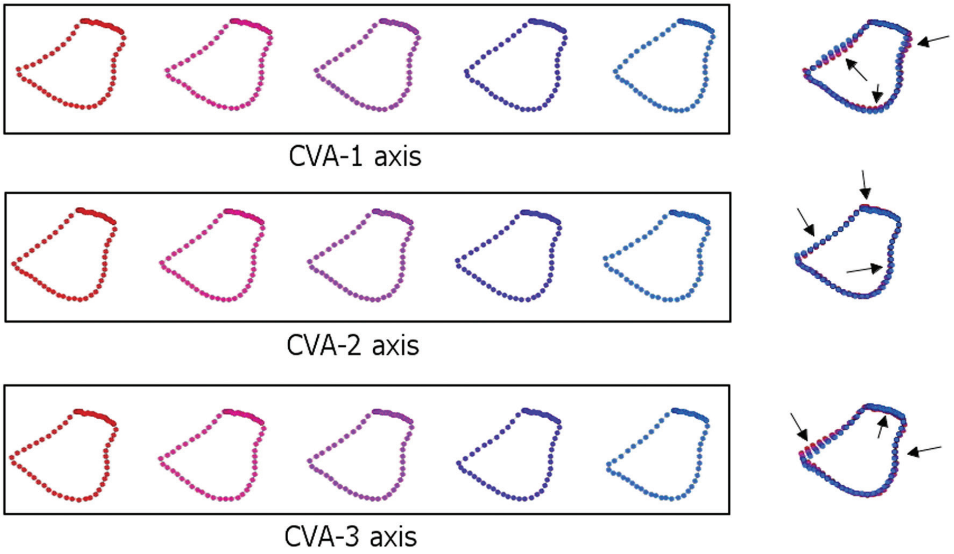

Figure 6. Strobe models of five positions along the canonical variates indicated in Fig. 5. CV-1, CV-2, and CV-3 axes account for 79.5% of the observed between-species shape variation. Landmarks and semilandmarks are superimposed in the figure to the right of each sequence to express the magnitudes and directions (arrows) of shape trends. In all models, the mesial margin of the coxa is depicted to the left, the lateral margin to the right.

the first three CV axes were repeated along the four remaining axes. Models calculated for CV-1 show clear and pronounced variation from the coxofemoral condyle to the fourth spine-bristle (i.e., the course of the lateral margin of the coxa), which trends from distinctly concave to slightly convex, corresponding to a relative increase in coxal width. In addition, the posteromedial coxal edge also indicates a slight variation, further increasing the coxal width in its posterior part. In contrast, a small variation in the anterior medial margin of the coxa involves a reduction in the width of the coxa in its anterior part.

The CV-2 models also exhibited variation in the exterior/lateral margin of the coxa. However, variation here is (expectedly) more subtle than in CV-1 and the shift oriented in the opposite direction. The anterior margin of the coxa of CV-2 illustrates some variation anteriorly, particularly between the second and the fourth spine bristles, reducing the coxal length. In contrast, there is a slight variation in the posteromedial edge of the coxa that increases the coxal width in its posterior part.

The CV-3 model series identifies changes in three different areas of the coxa: (1) extension towards the coxofemoral condyle becomes more prominent; (2) the anterior edge exhibits a variation similar to CV-2 but in reverse, slightly increasing the coxal length; (3) the coxal interior margin exhibits a shift that reduces the coxal width. The overall coxal shape displays a slight increase in relative length and a reduction in width along the CV-3 axis.

Using the CV models from Figure 6 and the canonical variates space (Fig. 5) to interpret these results taxonomically, it can be seen that *Parascutigera guttata* has a relatively narrower forcipular coxa than the other sampled taxa, exhibiting a concave exterior margin with a very pronounced extension to the coxofemoral condyle. Alternatively, *Thereuopodina queenslandica* and *Dendrothereua linceci* display relatively wider coxae. *Thereuopodina*, however, exhibits a convex exterior margin, whereas *Dendrothereua* has a slightly shorter coxa with a concave exterior margin. In contrast, most specimens of *Thereuonema microstoma* and *Scutigera coleoptrata* exhibit a long and narrow coxa; *Thereuonema* exhibits a slight concave exterior margin and more pronounced coxofemoral condyle, while *Scutigera* has a straight exterior coxal margin. *Allothereua maculata* specimens are characterized by intermediate shapes along the axes. As reflected by their discriminant function results (Tables 3, 4), *Thereuopoda longicornis* and *Sphendononema guildingii* each display substantial variability in forcipular coxal shape.

Table 3. CV discriminant function analysis showing the percentage of specimens that were correctly assigned to their original species.

Taxa	*A. maculata*	*D. linceci*	*P. guttata*	*S. coleoptrata*	*S. guildingii*	*T. longicornis*	*T. microstoma*	*T. queenslandica*	Total Correct	% Correct
A. maculata	11	0	0	0	0	1	1	0	11	85
D. linceci	2	9	2	0	0	0	0	0	9	69
P. guttata	0	0	17	0	0	1	0	0	17	94
S. coleoptrata	1	0	0	9	0	0	2	0	9	75
S. guildingii	0	1	0	0	3	0	0	1	3	60
T. longicornis	0	2	1	1	1	13	0	1	13	68
T. microstoma	1	0	1	0	0	0	11	1	11	79
T. queenslandica	0	1	0	0	1	2	0	10	10	71
								Total Correct	**83**	**77**

Table 4. CV discriminant function of jackknife analysis showing the percentage of specimens that were correctly assigned to their original species.

Taxa	*A. maculata*	*D. linceci*	*P. guttata*	*S. coleoptrata*	*S. guildingii*	*T. longicornis*	*T. microstoma*	*T. queenslandica*	Total Correct	% Correct
A. maculata	9	0	0	1	0	1	2	0	9	69
D. linceci	4	6	2	0	1	0	0	0	6	46
P. guttata	0	0	16	1	0	0	1	0	16	89
S. coleoptrata	1	0	0	5	1	1	3	1	5	42
S. guildingii	0	0	1	0	2	0	0	2	2	40
T. longicornis	1	2	1	1	1	11	1	1	11	58
T. microstoma	2	0	1	0	0	0	10	1	10	71
T. queenslandica	0	1	0	0	2	2	2	6	6	46
								Total Correct	**65**	**61**

Discussion

The morphometric results obtained from the Procrustes PCA of these landmark-registered semi-landmark outline data and the CVA models back-projected in the PC space suggest that forcipular coxal shape differs significantly between taxa at various taxonomic levels. This character complex is clearly valuable in the assessment of scutigeromorph systematics because the group has historically been classified on the basis of a small number of taxonomically informative characters (principally exoskeletal prominences on the tergal plates and female gonopod shape). Our finding that specimens can for the most part be assigned to species with a high degree of accuracy (Table 3) substantiates a prediction by Würmli (1974) that coxal shape contains taxonomic information. Until now, however, this variation had not been quantified or harnessed taxonomically or phylogenetically. The outlines and landmarks illustrated in Figure 2 may possibly form the bases for future analysis of coxal differences.

Controversies over the status of particular species, and indeed the species concept applied across the group as a whole, are informed by the results of this study. For example, the geographically widespread *Thereuopoda longicornis* shows a variable coxal shape and, as a result, a relatively poor capacity for assigning specimens accurately, with 68% correct discrimination of the training set (Table 3). Furthermore, coxal shape variability in *T. longicornis* has a discernible geographical pattern (Fig. 3), with specimens from each sampled southeast Asian region plotting near each other. Verhoeff (1905, 1937, and elsewhere) distinguished an array of nominal species of *Thereuopoda* that were later synonymised into *T. longicornis* by Würmli (1979). This difference in the number of valid species (as extreme as 26 species versus one) reflects, at least in part, the observation that some characters employed at the species level by Verhoeff were subsequently found to have overlapping variation as sample sizes were increased, and in part reflects a historical shift towards species being seen as polymorphic, geographically widespread entities by later 20th century taxonomists. The coxal shape differences observed between geographic groups of *T. longicornis* (specimens assigned to their correct geographic group with 89% accuracy; see Table 2) suggest that some of the subjective synonyms of *T. longicornis* may actually include valid species.

Another species with a comparably wide geographic range and complex taxonomic history, *Sphendononema guildingii*, likewise has a comparatively poor capacity to be discriminated (Tables 3, 4). The family Pselliodidae has long been identified as a high-ranking clade (Verhoeff 1904; Würmli 1978, 2005; Edgecombe and Giribet 2006). Our CVA models (Fig. 6) show that although *Sphendononema* has a relative short and wide forcipular coxa with a convex exterior margin, it is not readily distinguished from some members of Scutigeridae on coxal shape alone. Current taxonomic practice (including species identifications in this study) follows Würmli (1978) in placing many (22) nominal species in synonymy with *S. guildingii*, though some aspects of variation (e.g., female gonopod variability explored by Bolton et al. 2009) suggest the presence of multiple species. The low discriminant function scores for *S. guildingii*, in spite of a small sample size, would be consistent with a mixed-species sample.

Würmli (1973b) and Würmli and Negrea (1977) united *Scutigera linceci* as a single geographically widespread species which was later recognised as multiple species of *Dendrothereua* by Edgecombe and Giribet (2009). The latter regarded it as 'exceedingly doubtful' that *D. linceci* constitutes a single species because specimens from the limits of the geographic range (Costa Rica versus the southern U.S.) had molecular sequence divergences that greatly exceeded those in uncontroversial morphospecies. Although no significant infraspecific coxal differences can be related to geography, pooling specimens from each of Mexico (Guerrero), Guatemala and Hispaniola (Appendix, Fig. 1 therein), both the raw and jackknifed discriminant function scores (69% and 46%, respectively) indicate that a relatively low percentage of specimens are correctly assigned to this species (Tables 3, 4). Coxal shape does not provide strong corroboration for *D. linceci* being a single species.

The small sample size for some of the geographic groups within *Scutigera coleoptrata* prohibits statistical testing of whether infraspecific coxal shape differences can be related to geography. Würmli (1973a, 1977) revised the species-level taxonomy of *Scutigera*, proposing the synonymy of several species with *S. coleoptrata*, consistent with the view that *S. coleoptrata* is a synanthropic species throughout large extents of its geographic range, as well as with short molecular branch lengths between specimens from populations in different parts of that range (Edgecombe and Giribet 2009). The specimens studied here include native parts of the species' distribution (Italy, Greece, Madeira, Algeria) as well as introduced parts (St. Helena, Bermuda). The assignment of specimens to this species with 75% accuracy for raw CVA scores (Table 3) does not appear to be a stable result based on the poor capacity for identification in CVA jackknife tests (Table 4).

In contrast to the variability discerned in the species discussed above, some species, notably *Parascutigera guttata* and *Thereuonema microstoma*, display consistently distinct coxal shapes (Table 3) and these results are stable when subjected to a jackknife test (Table 4). The diagnosability of these species with respect to coxal shape is in agreement with recent classifications that have established *P. guttata* (Edgecombe and Giribet 2009) and *T. microstoma* (Stoev and Geoffroy 2004) as valid species. Both species have narrow forcipular coxae, but *Parascutigera guttata* has a pronounced concave exterior margin with a distinct projection of the coxofemoral condyle whereas *Thereuonema microstoma* exhibits a longer coxa with a straighter exterior margin. It should be noted that the high discriminant function score for *T. microstoma* might be expected to decrease were more specimens from distant parts of its geographic range included; nearly all specimens used in this study were from a small part of its total distribution, in the Sudan.

Conclusion

Geometric morphometrics of forcipular coxal shape indicates that these structures contain taxonomic information at the species level. Discriminant function analysis indicates that a majority of specimens of all eight species sampled in this study were assigned correctly according to their established taxonomy, in several cases with a high degree of accuracy. This investigation also demonstrates that morphometric approaches and CVA modelling procedures can be of considerable use in the analysis of subtle morphological features, and can support a wide variety of comparisons between groups at different taxonomical levels even in character systems that had been opaque to qualitative analysis, such as the forcipular coxae of Scutigeromorpha.

Acknowledgements

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Appendix 1

Voucher data for specimens use din morphometric analyses and supplementary figures of Canonical Variates scatterplots. File format: Adobe Acrobat (pdf) file.

Explanation note: Information on voucher specimens used in this investigation and infraspecific coxal shape data for *Dendrothereua linceci*.

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IN MEMORIAM

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Memories of Cas Jeekel, friend, colleague, and role model

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Nearly everyone has experienced an event that exerted a profound influence on some aspect of their life; often the full impact of that milestone is not fully appreciated until much later through the filter of retrospection. The first such event that established the course of my own scientific career occurred in November of 1946, when, searching for some group of animals to investigate, I attempted to identify some julid millipeds and discovered that the only comprehensive resource on North American species was published in 1893. The second came in the form of a letter dated 20 August 1949 from a certain C. A.W. Jeekel at the Amsterdam Zoological Museum, introducing himself as a beginning student of myriapods, and inquiring about the availability of certain type specimens in the U.S. National Museum. Thus began an exchange of ideas and information that evolved into a personal friendship that endured for more than 60 years, and resulted in reshaping my approach to taxonomy.

Up to that point in time, much of the published work on Diplopoda consisted of opportunistic descriptions of new taxa with scant attention to any form of synthesis. I was still under the malign influence of R.V. Chamberlin, an exemplar of minimal taxonomy, and my new friend in Holland was still publishing isolated descriptions, albeit with substantial amplification of his actions. However, my conversion to a different approach was catalyzed by his two 1951 papers about *Tectoporus* and *Sphaeropoeus* which showed that useful progress could be made by combining descriptions of new taxa with analysis of relevant published information. Maybe a piecemeal measure, but certainly an improvement and guide for the future work which I determined to adopt.

In 1960 I began the first of my sequence of visits to European museums searching for the types of enigmatic ancient species. After London and Tervuren, my next stop was Amsterdam, and since the KLM intown terminal was only a few minutes from the address which I had been given, I was soon looking up a steep narrow flight of stairs to the welcoming greeting and broad smile of a tall slender young man: after 11 years a Jeekel in the flesh. What ensued thereafter we later defined as a "marathon dialogue" that lasted three days and nights, fueled by many cups of coffee and punctuated by long ambulatory conversations around the city. Starved for such an exchange we exchanged views on research interests, and discussed the work of our predecessors and few contemporaries, finding that our views coincided closely on all points (admiration for Brolemann, Cook, and Pocock, for instance, despair over certain others). Cas showed me his accumulation of elegantly illustrated research projects, many of which only appeared as parts of his great *Myriapod Memoranda* series 40 years later.

He lived at the time in an archetypical bachelor two-room flat crowded with his already extensive collection of separata and books, and to my surprise, the evidence of his interest in music, particularly of New Orleans jazz: instruments: a large collection of 78 rpm recordings, and many books about prominent jazz musicians. He was an active member of a student ensemble, but even at that time music was being gradually preempted by myriapods. Regrettably we devoted little time to exchanges about personal backgrounds. I only learned that he had been born in Medan, Sumatra, and his family moved back to Holland when he was still young. But during that first visit the initiative for the *Nomenclator* was developed, and that mythical estimate of 80 000 extant species was contrived.

At the time he held a position in the Zoological Museum and was librarian for the Dutch Entomological Society. I was interested to learn to that although he passed the Rijksmuseum every day on the way to work, he had never entered that celebrated institution until he thought that his foreign visitor should be exposed to the "Night Watch" and arranged a detour to see it on one of our excursions.

Subsequent visits to Europe always included a stop-over in Amsterdam to discuss (de-brief!) the more interesting results of my delving in neglected collections and especially to follow the progress of the *Nomenclator Generum et Familiarum*. During this time, and even earlier, he had made his own pilgrimages to the museums in London, Genova, Tervuren and Geneva basically to examine material of paradoxosomatids. Curiously he never visited the great historically important collections in Munich, Berlin, and Vienna, nor endeavored to borrow material from them.

Out of a sense of filial obligation, Cas remained unmarried for many years in order to look after his aging mother. Eventually relieved of this responsibility, he encountered two major events that changed his life substantially. In early 1969 he became director of the Zoological Museum, a position assumed with much reluctance, and only because the only other likely candidate would have been "disastrous". This new status greatly improved his standard of living, but at the expense of his research and health: the stresses of administration resulted in chronic headaches and later a painful disorder of the nerves in his face. Nonetheless he produced

C.A.W. Jeekel at home, 1979. Image courtesy L.A. Pereira.

several papers every year during this period and completed the manuscript of the *Nomenclator*.

The second event was in every way a far happier one. In March of 1970, to use his words "I took the big step and married a lovely widow..." A. M. (Jeanne) Rijvers (known to attendees at CIM meetings as "Sjan") was the catalyst that brought about a virtual metamorphosis in Cas' life. I know he would not object to my sharing details of this milestone event, a remarkable and improbable story in itself, abstracted from his own account. On the bus ride to work one morning, he picked up a newspaper left behind by another passenger, and for what was surely the first time in his memory, looked at the section devoted to the promotion of new social interactions. From this unlikely departure, he selected a "woman looking for a man" entry that looked interesting and to his own astonishment, initiated the contact that proved to be the major milestone in his life. The word "destiny" might occur to the romantically inclined.

Sjan was the wife of a young school teacher in the Indies, who had moved to Holland with her two daughters following his untimely death. The axiom that "opposites attract" was never more true: it would be difficult to imagine two people so different in personalities, yet in practice so compatible. Younger, outgoing, and vivacious, she complemented and ameliorated Cas' sedate decorum. She became a skilled collector, and accompanied him to congresses as well as excursions to Malta, North Africa, United States, and Australia. They occupied a progression of homes in Heiloo, Bergen an Zee, and Oisterwijk, each more elegant than the last, each beautifully landscaped and planted (on field trips they collected stones for the gardens as well as myriapods). Each of my European circuits during the 1970s and 1980s typically ended with a few days at the Jeekels' home, the technical discussions conducted in such a gracious and relaxing ambience that I coined the term "The Lotus Garden" with reference to a similar experience enjoyed by Ulysses on his long return from Troy to Ithaca. That their final residence had to be in such different and constrained quarters was certainly a hardship for both. I was never able to visit during that time, perhaps as well that my last images of them would be in context of their Oisterwijk residence.

The extent to which we agreed on points of classification and nomenclature was remarkable. From the earliest days we knew that the era of random descriptive naming was over, and the direction of the future had to emphasis both janitorial work in cleaning up the inherited mess of bad taxonomy and nomenclatorial anarchy, and whatever synthesis could be accomplished with the available materials. I think the only things we disagreed on were pretty trivial (he preferred the spelling *Pollyxenus*, we parted company in several cases of generic typification, and he felt that the paradoxosomatid gonopod was generalized rather than derived). At no time was there ever an instance of verbal disharmony. We stated our opinions and ideas, to be accepted or not after reflection on their possible merits. Mentioning a visiting colleague who had brought some drawings of dubious quality, Cas reported "I did not criticize his drawings but merely put mine next to his, while explaining my ideas. . ." with the hope that such comparison might have a desired influence. He fully understood the fact that so much of taxonomy represents simply subjective evaluation of qualitative information, and was not readily amenable to being objectified. Needless to say, data matrices and cladograms were not part of his working protocols.

For me Cas Jeekel was an admired colleague with an overtone of role model and the only person who I conceded to be my master in our field of interest. His extensive research and publication, above all the monumental *Nomenclator*, set the standard for milliped systematics in the era that is now ending, and will continue to be consulted in the next one. *Ave atque vale, Magister et amicus*!

Richard L. Hoffman
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RESEARCH ARTICLE

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A remarkable case of mosaic parapatry in millipedes

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Abstract

The parapatric boundary between *Tasmaniosoma compitale* Mesibov, 2010 and *T. hickmanorum* Mesibov, 2010 (Polydesmida: Dalodesmidae) in northwest Tasmania was mapped in preparation for field studies of parapatry and speciation. Both millipede species can be collected as adults throughout the year, are often abundant in eucalypt forest and tolerate major habitat disturbance. The parapatric boundary between the two species is ca 100 m wide in well-sampled sections and ca 230 km long. It runs from sea level to 600-700 m elevation, crosses most of the river catchments in northwest Tasmania and several major geological boundaries, and one portion of the boundary runs along a steep rainfall gradient. The location of the boundary is estimated here from scattered sample points using a method based on Delaunay triangulation.

Keywords

Diplopoda, Polydesmida, Dalodesmidae, millipede, Australia, Tasmania, parapatry, boundary estimation, Delaunay triangulation

Introduction

Millipedes commonly form lineage mosaics (Mesibov 2003c), in which each species has a discrete range that overlaps very little or not at all with the ranges of other species in the same genus (Shelley 1990a, 1990b). Tasmanian examples in the dalodesmid Polydesmida are found in the genera *Atrophotergum* Mesibov, 2004 (Mesibov 2004), *Dasystigma* Mesibov, 2003 (Mesibov 2003a), *Gasterogramma* Jeekel, 1982 (Mesibov 2003b), *Lissodesmus* Chamberlin, 1920 (Mesibov 2006) and *Tasmaniosoma* Verhoeff, 1936 (Mesibov 2010).

It is not yet known how the tight parapatry seen in these mosaics originates and is maintained (Mesibov 2003c). What is apparent is that parapatric boundaries between millipede species are not necessarily congruent with environmental boundaries. In Tasmania the boundaries cross rivers and traverse different underlying geologies, soil types, local climates and vegetation types.

I document below a remarkable parapatric boundary of the 'environmentally incongruent' kind between two congeneric millipedes in northwest Tasmania. I also demonstrate a simple analytical method for estimating and visualising the location of a parapatric boundary from point localities.

Materials and methods

Millipede species

The endemic Tasmanian species *Tasmaniosoma compitale* Mesibov, 2010 and *T. hickmanorum* Mesibov, 2010 were named, described and provisionally mapped in Mesibov (2010). Both are ca 15 mm long as adults. The two species are very similar in appearance, but live and freshly preserved males and females can usually be identified by a difference in colour: *T. hickmanorum* are red-brown, while *T. compitale* are yellow-brown with a distinct light patch just under the lateral margin of each paranotum (Fig. 1 in Mesibov 2010). Unfortunately, live colouring in both species is somewhat variable, and some females cannot be confidently assigned to species. For this reason, locations for females not associated with males are marked separately in the map Figures, below, and parapatric boundary estimation is based on males only.

Colour in the two species fades quickly in alcohol. Preserved females cannot be identified by colour after a few months, and long-preserved museum specimens can only be identified by examining the gonopods of mature males.

Millipede sampling

For the present study I collected *T. compitale* and *T. hickmanorum* by hand during the daytime at 351 sites in northwest Tasmania between July 2009 and August 2011. Adults are night-wandering and can be found sheltering during the day under loose fragments of bark on dead tree stems and branches, under flat pieces of bark and woody litter on the ground, and within narrow, curled lengths of fallen bark from eucalypt crown branches. At most sites adults were collected within 30 minutes, but at sites where *Tasmaniosoma* spp. were uncommon I extended the search to ca 1 hour. Several sites were searched on more than one field day before *Tasmaniosoma* spp. were found, and a number of specimens were kindly provided by other collectors during the study period. All specimens were preserved in 80% ethanol and deposited as registered lots in the Queen Victoria Museum and Art Gallery, Launceston, Tasmania.

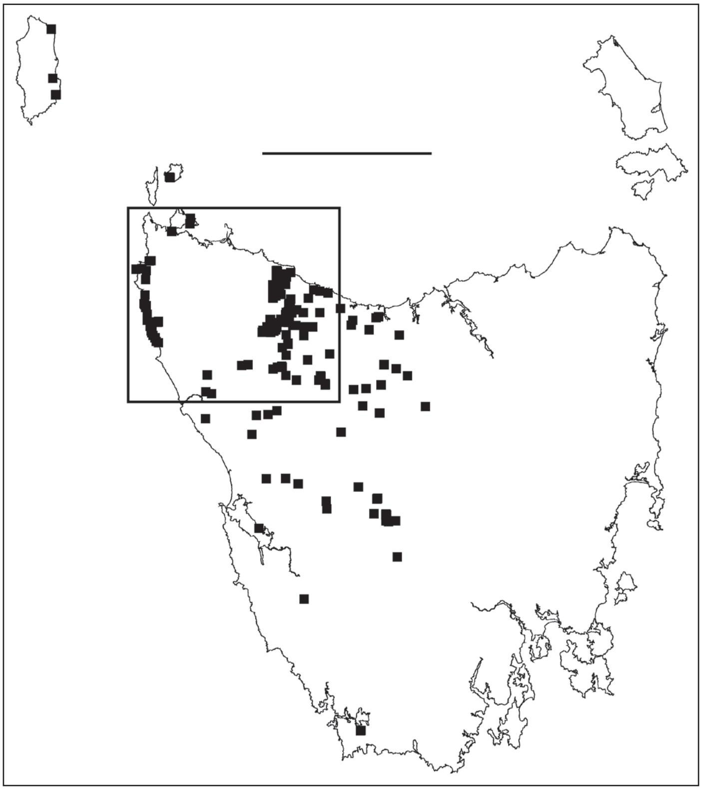

Figure 1. Localities for male *Tasmaniosoma hickmanorum*. Scale bar = 100 km. Rectangle indicates map extent in Figs 2–6.

Sites were located in the field with a handheld GPS unit, or later the same day by reference to Google Earth. Because I collected specimens over an area up to ca 1 ha at each site, the uncertainty associated with each locality was recorded as ±25 m or ±50 m as appropriate.

Note: All known specimen records for *T. compitale* and *T. hickmanorum* are listed in the Appendix. The reader should refer to this Appendix for locations of placenames and other geographic features mentioned in the Results and Discussion sections. Site locations can be plotted from Appendix data either as UTM grid references or as latitude/longitude in GIS, or in Google Earth or other spatial data browsers using the included KML files.

Boundary estimation

A parapatric boundary can be marked on a map by drawing a line equidistant from localities for each of the two species near the boundary. A 'halfway between' line of this kind is an appropriate estimator of the true location of the boundary if sampling localities are few and the map scale is coarse.

The present study generated hundreds of species localities and I was interested to trace the path of the main parapatric boundary (see below) at a scale appropriate to the spatial intensity of the sampling. I first generated a Delaunay network for male localities in the area of interest using the Delaunay triangulation tool in Quantum GIS version 1.4.0 (http://qgis.org/); see Fig. 4 and its caption for more details. I then deleted from the Delaunay polygon shapefile all triangles which had the same species at all three vertices. For clarity, I also deleted triangles running seawards from the two coastal ends of the boundary. The remaining set of 163 polygons (Fig. 5; see also its caption) consisted of triangles with one species at two of the vertices and the other species at the third. Finally, I generated centroids for the 163 triangles (Fig. 6) using the freeware Center of Mass extension (Jenness Enterprises, Flagstaff AZ, USA, http://www.jennessent.com/) for ArcView 3.2 GIS (ESRI, Redlands CA, USA).

Each of the 163 centroids is a point estimate for the location of the parapatric boundary. Where sampling localities are close together, the centroids approximate a line; see the eastern section of the boundary in Fig. 6. Where sampling localities are far apart, the centroids are diffusely distributed. The method used here is similar to triangulation wombling for scattered point data (Fortin and Drapeau 1995), but tentative 'boundary elements' have not been generated by joining centroids. The 'cloud of centroids' in Fig. 6 shows the relative uncertainty in estimating the location of the boundary from place to place, something which a hand-drawn or computed line cannot easily do.

Spatial data sources

The GIS layers used in Fig. 7 for elevation contours, major streams, generalised geology and rainfall isohyets were sourced from the spatial data library of the Tasmanian state government (http://www.thelist.tas.gov.au) through the Queen Victoria Museum and Art Gallery.

Results

Millipede sampling overview

Adult *Tasmaniosoma* spp. were collected in every month of the year during the study period, but were much harder to find in the Tasmanian summer, December to March.

In northwest Tasmania, *T. compitale* and *T. hickmanorum* appear to be most abundant in natural forest and woodland of the regional *Eucalyptus* species, namely *E. brookeriana*, *E. delegatensis*, *E. nitida*, *E. obliqua*, *E. ovata* and *E. viminalis*. Both species can also be found in riparian or fire-sere stands of *Acacia dealbata* and *A. melanoxylon*. Both millipede species occur in eucalypt communities ranging from open forest with an iris ground layer (*Diplarrena* sp.), to regrowth forest with a dense understorey of small trees (*Pomaderris apetala* or *Leptospermum* spp.), to old-growth forest with an understorey of mature rainforest tree species (*Nothofagus cunninghamii*, *Phyllocladus aspleniifolius*, *Atherospermum moschatum*). Neither species is easy to find in old-growth *N. cunninghamii* rainforest or in heathland.

Populations of both *T. compitale* and *T. hickmanorum* appear to be tolerant of major habitat disturbance, including intense wildfire and clearfelling followed by burning and reafforestation. Both species are abundant in some plantations of *Eucalyptus globulus*, *E. nitens* and *Pinus radiata*, especially first-rotation plantations established on former native forest sites and plantations closely bordering native forest, including narrow remnant strips close to watercourses. Both species are also occasionally found in small (<0.1 ha) vegetation remnants on cleared farmland, e.g. an isolated eucalypt copse or a riparian strip of *A. melanoxylon* over blackberry (*Rubus fruticosus*) and other weed species.

I was unable to find either species in certain apparently suitable habitat patches, despite repeated searches during the two-year study period. These distribution gaps were mainly clustered in the southeast of the study area near Waratah and Guildford, but were also close to former *Poa* grassland/woodland sites near Oonah. Populations of *T. compitale* or *T. hickmanorum* were found at sites within a few kilometres of the gaps.

Main parapatric boundary

T. hickmanorum is widespread in western Tasmania but there is a ca 4000 km^2 gap in its distribution in the northwest (Figs 1, 2). The gap is filled by the distribution of *T. compitale* (Fig. 3). The main parapatric boundary between the two species is ca 230 km long (Fig. 6) and has been closely mapped along Jefferson Road southeast of Preolenna and Rebecca Spur 3 southeast of Temma. At these two locations the boundary is ca 100 m wide (Fig. 8). The boundary appears to be just as narrow along Leonards Road south of Henrietta, Lyons Road south of Lapoinya, the Murchison Highway southeast of Waratah and at the north end of Hellyer Gorge, Oonah Road west of Oonah and Robbins Island Road north of West Montagu. At these locations I was unable to collect enough males during the study period for reliable fine-scale mapping.

Boundary mapping is no longer possible near Wynyard, Redpa and other farming localities in northwest Tasmania. *T. compitale* and *T. hickmanorum* populations persist in native vegetation remnants in these areas, but are separated by intensively managed pasture and cropland. Mapping is also difficult along the southern and southwestern sections of the main parapatric boundary, which traverses unroaded, largely wild country.

Arthur-Hellyer ‘island’

A ca 40 km^2 ‘island’ of *T. hickmanorum* localities occurs in the upper reaches of the Arthur and Hellyer Rivers near Hellyer Gorge (Fig. 2), apparently enclosed by *T. compi-*

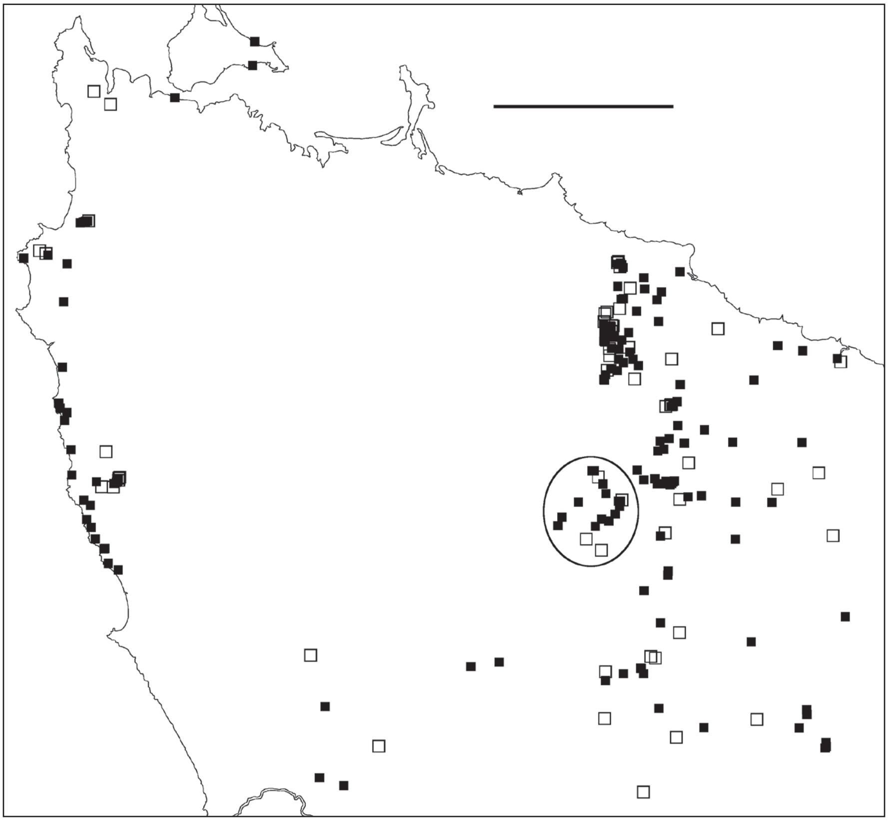

Figure 2. Localities for *Tasmaniosoma hickmanorum* males (filled squares) and females identified by colour (open squares). Ellipse encloses Arthur-Hellyer 'island' (see text for explanation). Scale bar = 25 km.

tale localities. The parapatric boundary of this 'island' has not yet been closely mapped on its eastern and northern sides, and the western and southern sections of the boundary (not yet located) are in unroaded, steeply dissected and thickly vegetated terrain.

Possible translocations

Males of *T. compitale* were found ca 20 km within the *T. hickmanorum* range at the Vale of Belvoir (Fig. 3). It is not yet known whether this is a naturally isolated occurrence or the result of translocation. The ca 500 ha of open grassland at the Vale has been grazed by cattle during the summer months for more than 100 years, and *T. compitale* may have been unintentionally introduced into the area by cattle-carrying trucks.

Translocations of *T. compitale* over shorter distances may also have occurred in intensively managed forest close to the main parapatric boundary. For example, I col-

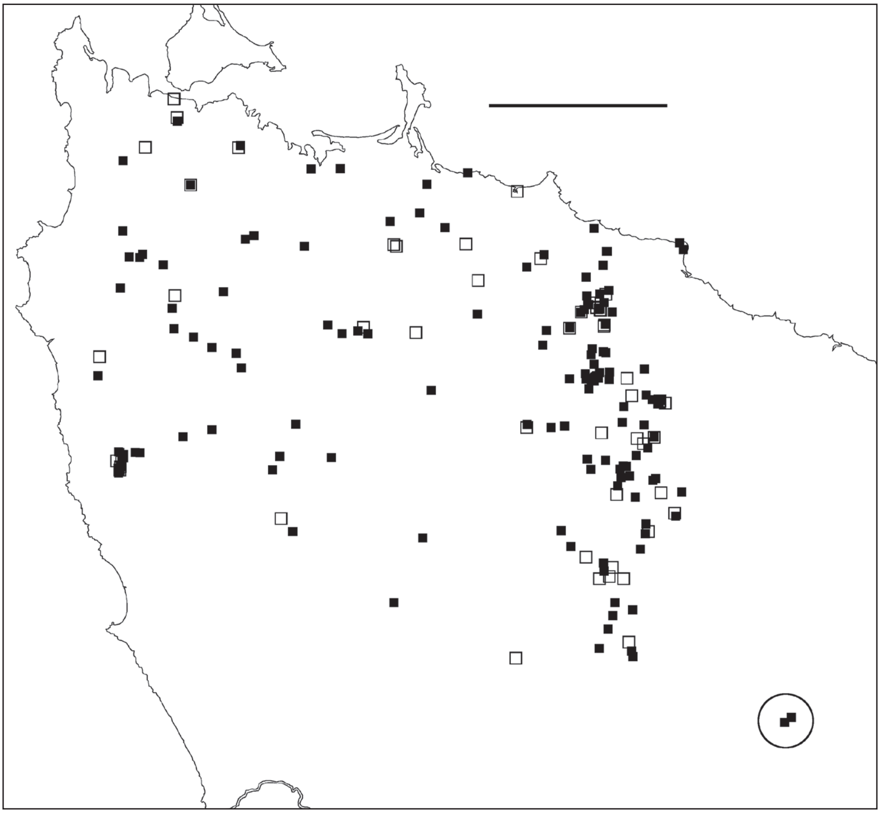

Figure 3. Localities for *Tasmaniosoma compitale* males (filled squares) and females identified by colour (open squares). Outliers (circled) are at the Vale of Belvoir. Scale bar = 25 km.

lected females of this species at three neighbouring sites along Ten Foot Track off Preolenna Road. The three sites are surrounded by *T. hickmanorum* occurrences and are close to a logging road used by log-carting trucks in recent years.

Co-occurrence

At two sites along the main parapatric boundary (Figs 4-6) I found males of *T. compitale* and *T. hickmanorum* within a few metres of each other, on Leonards Road south of Henrietta and on Talunah Road west of Hampshire. At another 11 sites close to the main parapatric boundary I found possible co-occurrences, but at least one species was represented only by females identified by colour. At two of the latter sites, both species were found sheltering under loose bark on the same fallen tree.

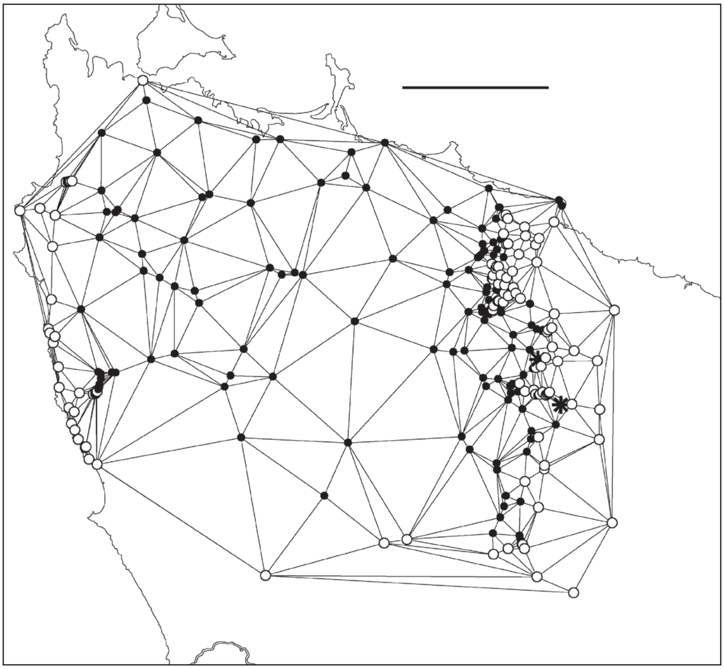

Figure 4. Delaunay triangulation of localities for male *Tasmaniosoma compitale* (filled circles), *T. hickmanorum* (open circles) and co-occurrences (stars). The set of localities used has been trimmed to the vicinity of the parapatric boundary, and *T. hickmanorum* localities in the Arthur-Hellyer 'island' and *T. compitale* localities at the Vale of Belvoir have been excluded. Scale bar = 25 km.

No evidence of hybrids

None of the males collected near the main parapatric boundary or the boundary of the Arthur-Hellyer 'island' had gonopods intermediate between *T. compitale* and *T. hickmanorum*, or were intermediate in live colouring.

Environmental incongruence

As shown in Fig. 7A, the main parapatric boundary rises from the north coast near Robbins Island (western section of the boundary) to 600-700 m at its southeast corner, then returns to the coast near Table Cape, ca 75 km from its starting point. It crosses most of the west coastal rivers north of Sandy Cape and the headstreams of both the major inland river systems in the region (Arthur and Pieman) before descending to the north coast; the northeast section of the boundary more or less follows the Flowerdale River (Fig. 7B). The parapatric boundary crosses numerous geological boundaries (Fig. 7C) and its eastern section runs along a fairly steep rainfall gradient (Fig. 7D), i.e. at right angles to isohyets.

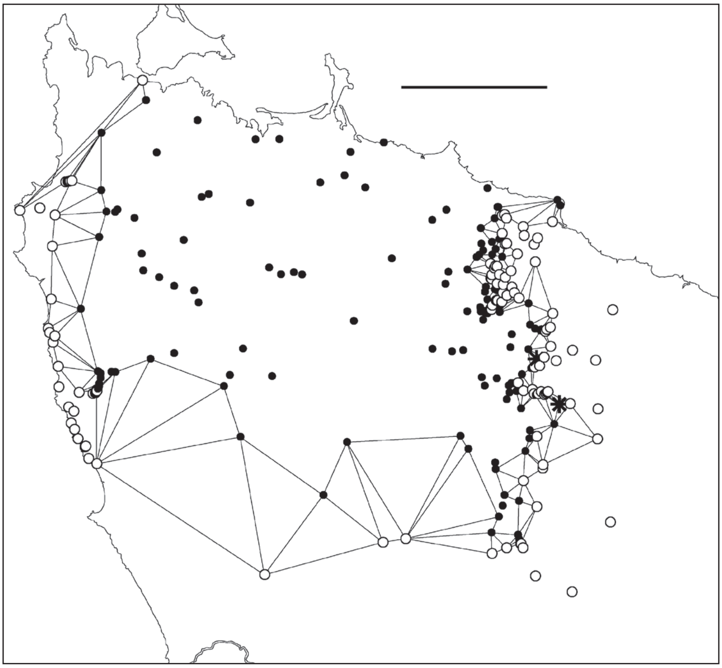

Figure 5. Edited Delaunay triangulation of localities for male *Tasmaniosoma compitale* (filled circles), *T. hickmanorum* (open circles) and co-occurrences (stars); see text for explanation. Scale bar = 25 km.

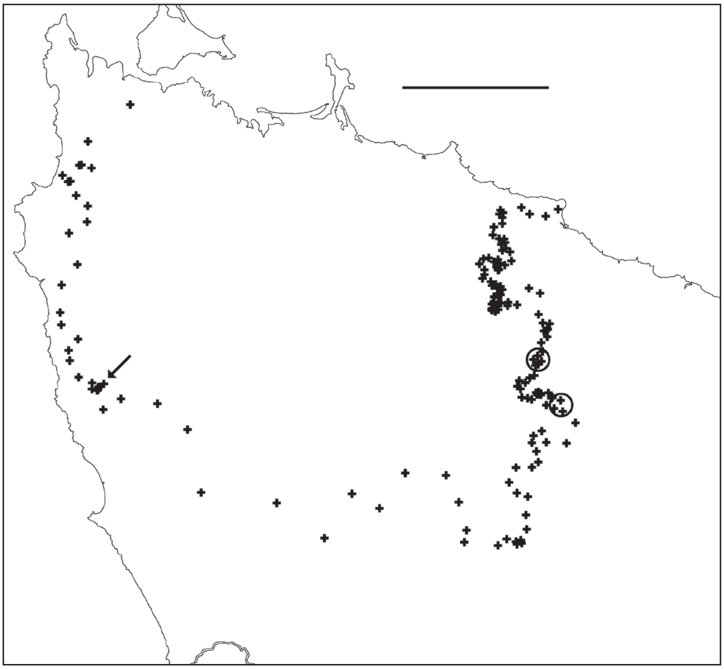

Figure 6. Centroids of triangles in Figure 5. Co-occurrences of *Tasmaniosoma compitale* and *T. hickmanorum* males are circled. Arrow points to area shown in Figure 8. Scale bar = 25 km.

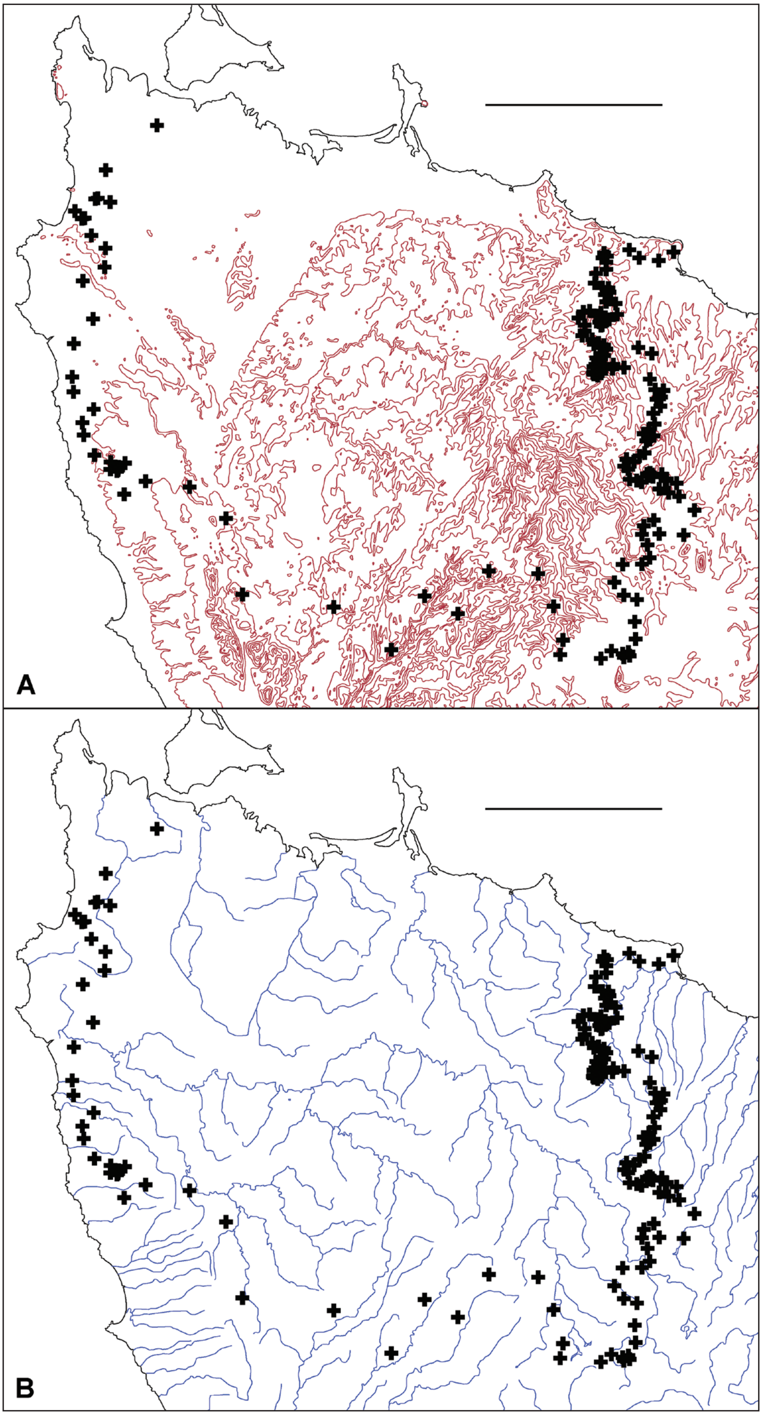

Figure 7A, B. Centroids from Fig. 6 superimposed on 100 m elevation contours **A** and principal rivers **B**. Scale bars = 25 km.

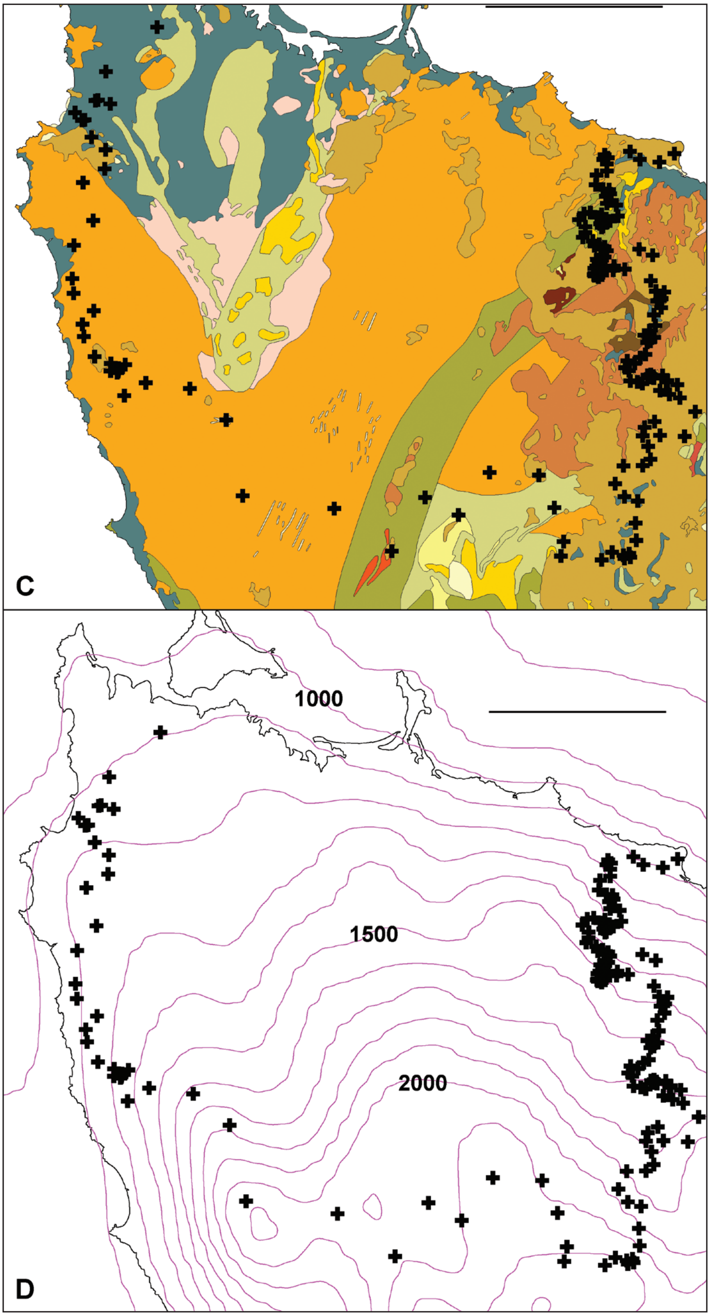

Figure 7C, D. Centroids from Fig. 6 superimposed on simplified bedrock geology **C** and mean annual rainfall isohyets, in mm **D**. In **C**, colours crossed by main parapatric boundary represent (anticlockwise from top left) Quaternary coastal sand and gravel (gray-blue), Precambrian siltstone and mudstone (orange), Precambrian metamorphics (green), Cambrian conglomerate and siltstone (gray-green), Tertiary basalt (light brown) and Permian glaciomarine sedimentary rocks (red-brown). Scale bars = 25 km.

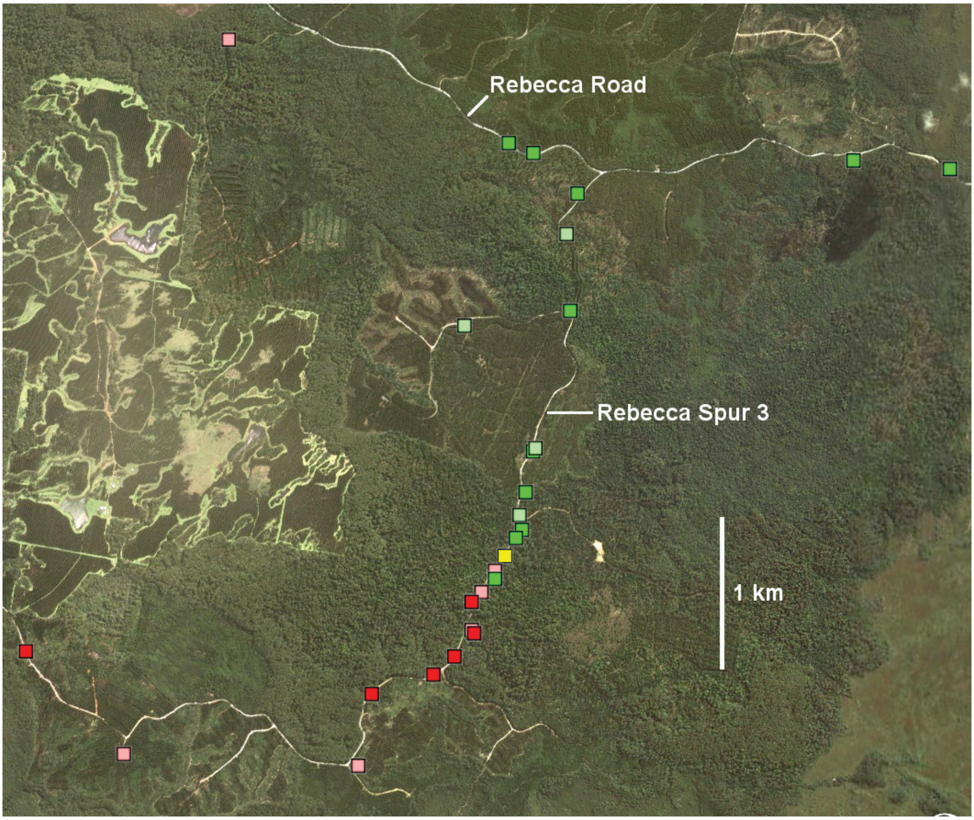

Figure 8. Rebecca Spur 3 area (marked with arrow in Fig. 6) in Google Earth image dated 11 October 2010. Markers: *Tasmaniosoma compitale* males (darker green) and females identified by colour (lighter green), *T. hickmanorum* males (darker red) and females identified by colour (lighter red), and possible co-occurrence of females identified by colour (yellow).

Both *T. compitale* and *T. hickmanorum* occur in a wide range of vegetation types close to the parapatric boundary. If there are major environmental boundaries which are spatially congruent with the biogeographical one separating the two species, they are not obviously related to topography, geology, climate or vegetation.

Discussion

The aim of this study was to map the boundary between the *T. compitale* and *T. hickmanorum* distributions as a knowledge base for future studies of parapatry and speciation. The parapatric boundary between these two species is the longest and narrowest I am aware of in the Australian millipede fauna. It is particularly well-suited to field study because much of it is easily accessed by all-weather roads, and because sections of the boundary run through little-disturbed tracts of native vegetation, including primary forest. The two millipede species are ecologically resilient and can be very abundant in eucalypt forest at lower elevations.

Several of the the largest and least disturbed patches of forest along the boundary are on public land managed by Forestry Tasmania, a government-owned forestry business. One such patch covers ca 12 km^2 on the upper Flowerdale River between Lapoinya and Preolenna Roads, on the eastern arm of the boundary. The forest is a mosaic of formal and informal reserves, production forest and plantation, with privately owned forest on the periphery. A similar but smaller mosaic is found along Rebecca Spur 3 and south of the Rebecca Road – Rebecca Spur 3 junction (Fig. 8) on the western arm of the boundary.

The parapatric boundary also crosses private land. Because the future of privately owned forest is less certain in Tasmania than that of formally reserved forest on public land, future field studies on suitable private blocks might be given a high priority. I was allowed access during the study period to a large private block near Henrietta, just north of the Takone Road. The Henrietta block carries even-aged eucalypt regrowth and populations of both *T. compitale* and *T. hickmanorum*.

An unanswered question is whether the two millipede species are well-distributed across heathland near the west coast. *T. hickmanorum* is abundant in shrubby coastal vegetation, and to the east *T. compitale* has been found close to scattered, small eucalypts in heath. As currently estimated (Fig. 6), the main parapatric boundary crosses ca 50 km of heathland in the formally reserved Arthur-Pieman Conservation Area.

Acknowledgements

I thank Matthew French, Daniel Lester, Renate van Riet and my wife Trina Moule for help with collecting, and Mark Alexander and Kevin Bonham for additional specimens. I am especially grateful to the many friendly farmers and other small-property owners in northwest Tasmania who gave me access to their properties for millipede sampling, and to the larger-property owners and managers who offered access through more formal protocols: Forestry Tasmania (represented by Nigel Foss), Grange Resources (Daniel Lester), Gunns Ltd (Robert Onfray) and the Van Diemens Land Company (Brent Anstis). This study was funded by the author.

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Appendix

Specimen records as of 8 August 2011 for *Tasmaniosoma compitale*, *T. hickmanorum* and unassigned females of these species.

Description. The compressed file contains complete records in the annotated CSV file 'Parapatry_records.csv' and separate KML files for males of the two species, females and juveniles of the two species (identified by colour), co-occurrences, and females not assignable to either species. Each KML record has an associated museum registration number and users should refer to 'Parapatry_records.csv' for more information on that record.

Citation: Mesibov R (2011) A remarkable case of mosaic parapatry in millipedes. In: Mesibov R, Short M (Eds) Proceedings of the 15th International Congress of Myriapodology, 18-22 July 2011, Brisbane, Australia. ZooKeys 156: 71–84. doi: 10.3897/zookeys.156.1893.app

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Two remarkable new species of Penicillata (Diplopoda, Polyxenida) from Table Mountain National Park (Cape Town, South Africa)

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Abstract

Two new species of the families Polyxenidae and Synxenidae, are described from Table Mountain National Park, South Africa. *Propolyxenus squamatus* **sp. n.** (Polyxenidae) has tergites I–X mostly covered by scale–shaped trichomes directed caudally, a character previously known only in Synxenidae. The structure of scale–shaped dorsal trichomes is different to that of the scales in *Phryssonotus* and *Condexenus* species. *Phryssonotus brevicapensis* **sp. n.** (Synxenidae) is the only known species of the genus *Phryssonotus* having 11 tergites, (including collum and telson) and 15 pairs of legs, as in *Condexenus biramipalpus* Nguyen Duy–Jacquemin, 2006. These two species therefore appear to occupy an intermediate position between *Phryssonotus* (12 tergites) and Polyxenoidea (maximum of 11 tergites).

Keywords

Diplopoda, Polyxenidae, Synxenidae, *Propolyxenus, Phryssonotus*, new species, scales, barbate trichomes, postembryonic development, South Africa

Introduction

Two new species of Penicillata from Table Mountain National Park (near Cape Town), South Africa, belonging to two different families, were collected in the same biotope: leaf litter of felled pine and fynbos.

The first species, represented by five specimens, belongs to the family Polyxenidae and the genus *Propolyxenus* Silvestri, 1948, created for *P. aegeus* Silvestri, 1948 from Rhodes (Pelecano) (Silvestri 1948). Silvestri distinguished *Propolyxenus*, with three transversal rows of trichomes per tergite, from the genus *Polyxenus* Latreille, 1803 which has only two rows. Condé and Nguyen Duy–Jacquemin (2008) united *Propolyxenus*, *Polyxenus* and *Typhloxenus* Condé, 1954 in the subfamily Polyxeninae Lucas, 1840, based on the structure of the telson. Silvestri's brief description was revised after an examination of diagnostic features of two adult syntypes of each sex by Short and Huynh (2010), who also gave a key to the species for the first time.

The second species, represented by 20 specimens, belongs to the family Synxenidae and the genus *Phryssonotus* Scudder, 1885 (a replacement for the preoccupied name *Lophonotus* Menge, 1854), whose type species is *L. hystrix*, a fossil found in Eocene Baltic amber (Menge in Koch and Berendt 1854, Scudder 1885, Condé 1954). Silvestri (1900) created the genus *Synxenus* Silvestri, 1900 for *S. orientalis* Silvestri, 1900 from Uruguay. Later on, Silvestri (1923) transferred to this genus *Polyxenus platycephalus* Lucas, 1846, from North Africa, Spain and Italy (Rasnitsyn and Golovatch 2004), and described *Synxenus capensis* Silvestri, 1923 from southern Africa (Stellenbosch) and *Synxenus novaehollandiae* Silvestri, 1923 from Australia (Mt Lofty, South Australia). The genus *Synxenus* was synonymized with *Phryssonotus* by Condé (1954). Silvestri's (1923) identification key included four of the six extant species now known; the main distinguishing characters in the key were the number of ocelli and barbate trichomes (2–6) arranged in a row near the anterior trichobothrium with a short funicule. Short and Huynh (2006) redescribed *P. novaehollandiae* and observed 11 ocelli from larva VI to adults. This was an improvement to Silvestri's key which nevertheless still concerned only four species. The two new species described in the present work show some additional and easily identifiable characters.

Abbreviations used:

MNHN	Muséum National d'Histoire Naturelle, Paris, France
SEM	Scanning Electron Microscopy
pl	pairs of legs

Material and methods

The material serving as the basis for the present work was obtained by hand collecting, pitfall trapping and litter sampling in pine and fynbos areas in Table Mountain National Park, South Africa (by Charmaine Uys). The material is preserved in 70%

ethanol. The bulk of this material, including the holotypes and several paratypes, has been deposited in MNHN.

For light microscopy, the specimens are mounted on slides in "Baume de Marc André". SEM micrographs were taken using a scanning electron microscope at the Zoology Department, University of Cape Town.

Systematics

Class Diplopoda de Blainville in Gervais, 1844
Subclass Penicillata Latreille, 1831
Order Polyxenida Verhoeff, 1934
Superfamily Polyxenoidea Lucas, 1840
Family Polyxenidae Lucas, 1840
Subfamily Polyxeninae Lucas, 1840

Genus *Propolyxenus* Silvestri, 1948

Propolyxenus Silvestri, 1948
Propolyxenus Silvestri, 1948: Nguyen Duy–Jacquemin and Geoffroy 2003: 100.
Propolyxenus Silvestri, 1948: Short and Huynh 2010: 13.

The genus is typical of the subfamily Polyxeninae, due to the structure of the telson, but shows more than 2 transverse rows of barbate trichomes on each tergite.

Propolyxenus squamatus Nguyen Duy–Jacquemin, Uys & Geoffroy, sp. n.

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http://species-id.net/wiki/Propolyxenus_squamatus
Figs 1–5

Type material. South Africa, Cape Town, Table Mountain National Park. Cecilia, Rooikat, site 12, felled pine, altitude 300 m, 33°59'43S, 18°25'22E, 4/X/2008, holotype adult female (no. 4); Cecilia, Rooikat, site 9, Afrotemperate forest, altitude 400 m, 33°59'34S, 18°25'12E, 4/X/2008, paratype adult female (no. 3); other paratypes: Kirstenbosch, Afrotemperate forest, site 5, altitude 400 m, 33°58'55S, 18°25'25E, 12/IX/2008, subadult female (12 pl) (no. 2); Cecilia, Spilhaus, Afrotemperate forest, site 13, altitude 400 m, 33°59'43S, 18°25'05E, 18/X/2008, larva with 8 pl (no. 5), all collected from leaf litter by Charmaine Uys and mounted on slides (MNHN).

Other material examined (non–type). Cecilia, Spilhaus, Fynbos, leaf litter, site 14, 33°59'53"S, 18°24'52"E, altitude 520 m, 18/X/2008, subadult male (12 pl) (no. 6), used for SEM.

Figure 1. *Propolyxenus squamatus* sp. n. subadult male, habitus, dorsal view, body length: 2.50 mm. (Photograph by M. Judson).

Etymology. The specific name refers to the scale–shaped tergal trichomes.

Diagnosis. Differs from all other congeners by the position and structure of the tergal trichomes: these flat scale–shaped trichomes cover the tergites and are different from the barbate trichomes of the lateral tufts, pleurites and head. They are observed for the first time in the family Polyxenidae. As in the family Synxenidae, they lie close to the tergites and are all directed caudally, but differ from those of Synxenidae in their shape and structure.

Description of two adult females. Measurements. Body length (without caudal penicil): 2.50 mm (holotype). Tarsus II length of 13th leg: 100 µm (holotype) and 105 µm (paratype).

Head (Fig. 2C). 6 ocelli on each side of which 1 antero–sternal (Figs 2D, 3F). Vertex with 1 pair of posterior tufts of 27+27 (holotype) and 24+25 trichomes (paratype), consisting of 3 rows, middle row with 12–13 trichomes (Fig. 3F); the distance between each tuft is about half their length.

Proportions of antennal articles as in Fig. 3A. Antennal article VI with 4 basiconic sensilla (Figs 2E, F, 3A): 2 anterior (a) shorter and thinner than the 2 posterior ones; the more posterior (p2) slightly thinner than the (p1) (Fig. 3B); 1 setiform sensillum (s) between anterior and posterior ones and 1 posterior coeloconic sensillum (c); antennal article VII with 3 basiconic sensilla, the anterior (a) slightly thinner than the others (Fig. 3C), 1 setifom sensillum (s) between the 2 posterior basiconic sensilla and 1 posterior coeloconic sensillum (c). The right antennal article VII of the holotype has 4 basiconic sensilla and 2 coeloconic sensilla, but this is recognizable as a regenerated antenna (as shown by Nguyen Duy–Jacquemin 1972) with 2 coeloconic sensilla on article VII and none on article VI (Fig. 3A).

3 trichobothria, arranged in a triangle, with the most internal (near posterior tufts of vertex) smaller than the 2 others (Figs 2C, D, 3F). Surface of labrum (Fig. 3G) with

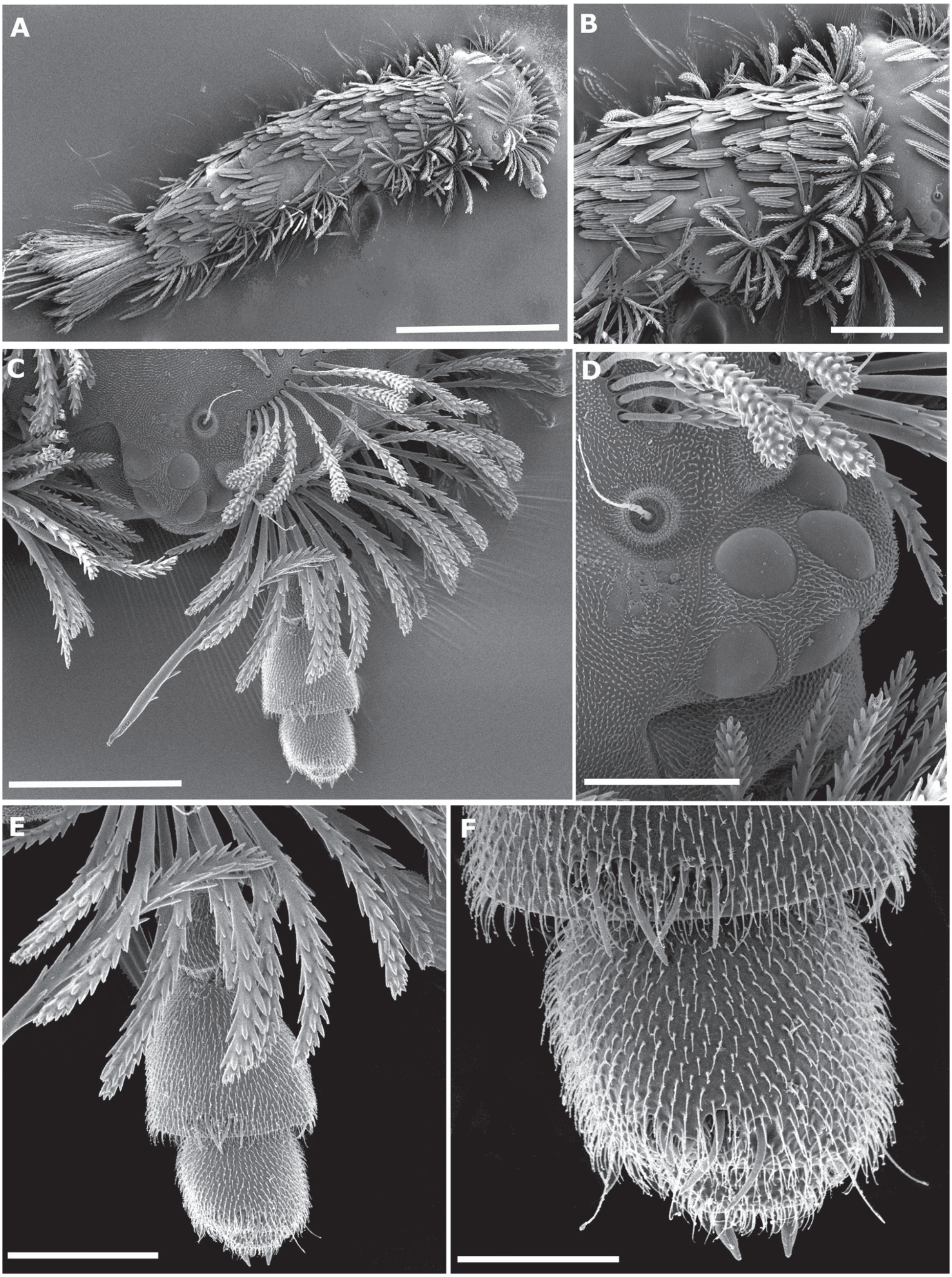


Figure 2. *Propolyxenus squamatus* sp. n. subadult male. **A** habitus, dorsal view **B** collum and tergites II–V, latero–dorsal view **C** right part of head with 2 trichobothria and antenna **D** ocelli and trichobothria **E** 4 last articles of right antenna **F** antennal sensilla on articles VI and VII, apical cones on article VIII. Scale bars: **A** 500 µm **B** 200 µm **C** 100 µm **D** 40 µm **E** 50 µm **F** 20 µm.

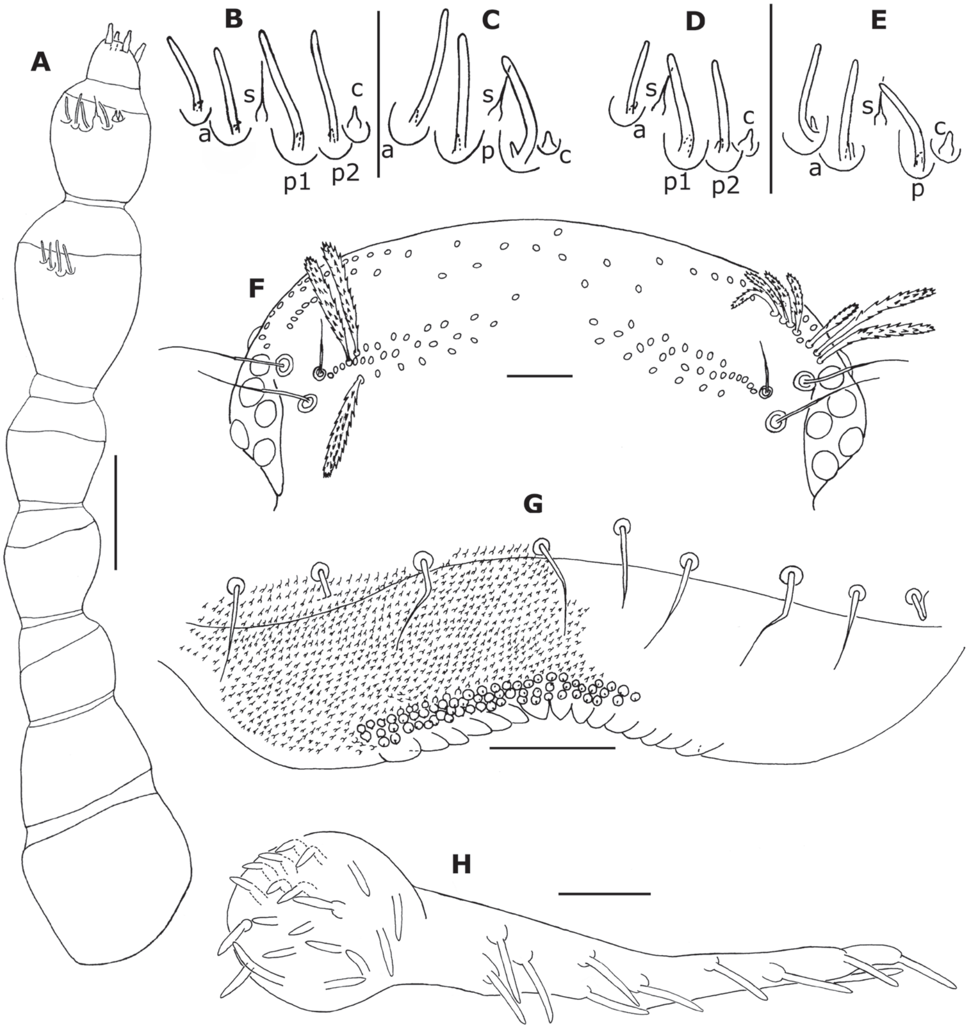


Figure 3. *Propolyxenus squamatus* sp. n. **A** right antenna of holotype female **B, C** sensilla of right antennal articles VI and VII of female paratype (no. 3) **D, E** sensilla of articles VI and VII right antenna of larva with 8 pl (no. 5) **F** vertex of holotype female **G** labrum of holotype female, papillae only represented on right part **H** left palp of gnathochilarium female paratype (no. 3). Abbreviations: **a** anterior basiconic sensillum **c** coeloconic sensillum **p, p1, p2** posterior basiconic sensillum **s** setiform sensillum. Scale bars: **A, F** 50 µm; others, 25 µm.

numerous small short cuspidate papillae; papillae of anterior 2 to 3 rows larger; 7+8 lamellate teeth on anterior margin (holotype: Fig. 3G), 8+8 (paratype); clypeo–labrum with 9 setae along posterior margin (Fig. 3G). Outer palp of gnathochilarium with 11 or 12 sensilla; middle palp with 19 or 20 sensilla (Fig. 3H).

Trunk (Fig. 2A): On each tergite (except collum, tergite X and telson) the trichomes are arranged in 3 rows and 2 lateral tufts (Figs 2B, 4A, B); each paired tuft connected by posterior and anterior rows of trichomes; middle row with more spaced trichomes (Fig. 4A). There are 2 types of trichomes. The flat trichomes, referred to as scales (sc) of the 3 rows are wider than barbate trichomes of lateral tufts (bt) and their shape and structure are different (Figs 2B, 4B, 5A–C); they look like the scales of Synxenidae by their position: all are directed caudally and cover the tergites I (with only a posterior row) to X (Figs 4A, B). Their structure is different from scales of Synxenidae (Figs 5A–E). The trichomes of the lateral tufts are longer and arranged in a bunch (Figs 2B, 4A, B (bt), C). Lateral protuberance of tergite I with 3 barbate trichomes (Fig. 4A).

Legs (Figs 4D–H): Naming of leg segments is after Brolemann (1935). Coxae I with 1 seta and coxae II with 2 setae; all other coxae without seta. All trochanters and prefemora with 1 seta; these setae having an oval base furnished with acute process at apex (Fig. 4F). All tibias (except 13) have 1 small seta tapered apically as shown in Figs 4D, E; other articles without seta. Tarsus II spine (Fig. 4H) longer than telotarsus (Fig. 4G): length of spine to claw ratio about 1.80. Telotarsus bearing an anterior process (ap) with a spinous projection longer than claw, 2 latero–anterior and posterior spiniform processes (t), posterior larger than anterior; posterior lamellar process (plp) thickened and basally pleated (Fig. 4G).

Telson (Figs 1, 2A): typical of genera *Propolyxenus*, *Polyxenus*, *Typhloxenus* (subfamily Polyxeninae). 21 (holotype) and 25 (paratype) dorsomedian barbate trichomes on caudal penicil. Hooked trichomes with 3 or 4 hooks.

Description of subadult female, 12 pl (no. 2). Measurements. Body length (without caudal penicil): 2.40 mm. Caudal penicil length: 0.60 mm. Tarsus II length of 12th leg: 112 µm.

Head: 6 ocelli on each side. Antennal article VI with 3 basiconic sensilla (Fig. 3D); antennal article VII with 3 basiconic sensilla (Fig. 3E). Surface of labrum with numerous small short cuspidate papillae; papillae of anterior 2 rows larger; 9+9 lamellate teeth at anterior margin. Outer palp of gnathochilarium with 11 sensilla; middle palp with 19 sensilla.

Trunk: Scales on tergites I-IX. Lateral protuberances of tergite I with 3 barbate trichomes.

Legs: Coxae I with 1 seta and coxae II with 3 setae; all other coxae without setae. All trochanters and prefemora with 1 seta. All tibias (except 11 and 12) have 1 small seta tapered apically; other articles without seta. Telotarsus bearing an anterior process with a spinous projection longer than claw, 2 latero–anterior and posterior spiniform processes; posterior lamellar process thickened and basally pleated.

Telson: 22 dorsomedian barbate trichomes of caudal penicil. Hooked trichomes with 3 or 4 hooks.

Description of a larva 8 pl (no. 5). Measurements. Body length (without caudal penicil): 1.80 mm. Tarsus II length of 8th leg: 110 µm.

Head: 6 ocelli on each side. Vertex with 1 pair of posterior tufts of 20+19 trichomes consisting of 3 rows, the middle row with 10 trichomes. Antennal article VI with 3 basiconic sensilla: 1 anterior shorter and thinner than the 2 posterior ones; the more posterior slightly thinner than the other; 1 setiform sensillum between anterior and posterior basiconic sensilla and 1 posterior coeloconic sensillum; antennal article

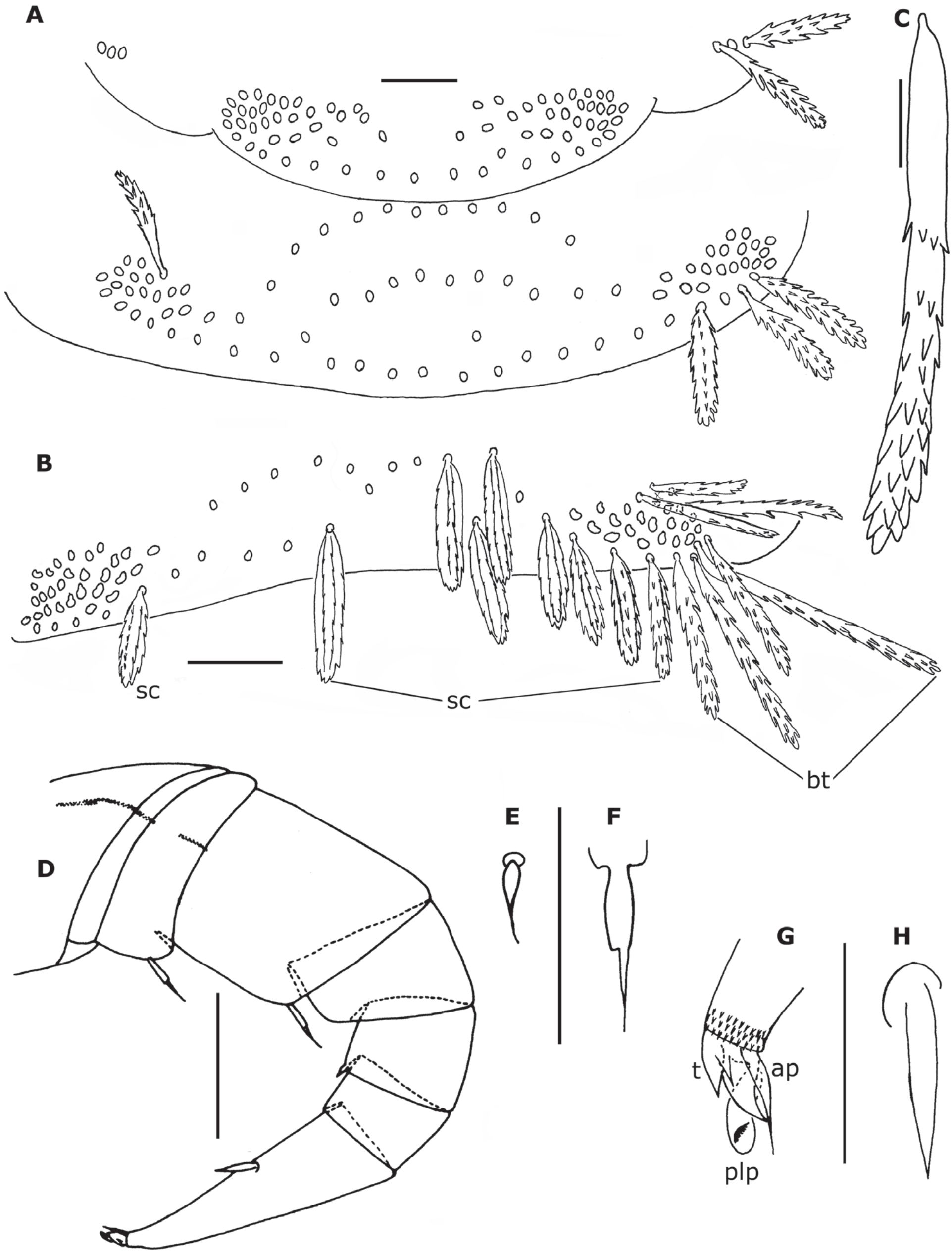


Figure 4. *Propolyxenus squamatus* sp. n. **A** Collum and tergite II of holotype female **B** tergite X of holotype female **C** barbate trichome of the right lateral tuft of tergite VII of holotype female **D** left leg 12 of holotype female **E, F** details of tibial and prefemora setae of the left leg 12 **G, H** telotarsus and tarsal II spine of right leg 13 of female paratype (no. 3). Abbreviations: **ap** anterior process **bt** barbate trichomes **plp** posterior lamellate process **sc** scale **t** latero–anterior and posterior teeth. Scale bars: **C, E–H** 25 µm; others, 50 µm.

VII with 3 basiconic sensilla, the anterior slightly thinner than the others, 1 setiform sensillum between the 2 posterior basiconic sensilla and 1 posterior coeloconic sensillum. 3 trichobothria, arranged in a triangle, with the most internal smaller than the 2 others. Surface of labrum as in adult females; clypeo–labrum with 10 setae along posterior margin. Outer palp of gnathochilarium with 12 sensilla.

Trunk: Trichomes arranged in 2 lateral tufts with 19 to 25 barbate trichomes connected by 3 rows of scales on tergites III–V and only 2 on other tergites. The tergites II–V have 22 to 31 scales, the collum 12 and the tergite VII 18. Lateral protuberance of tergite I with 3 (left) and 2 (right) barbate trichomes.

Telson: 18 dorsomedian barbate trichomes of caudal penicil. Hooked trichomes with 3 hooks (rarely 2 and 4).

Discussion. *P. squamatus* sp. n. is strongly distinguished from other species of the genus by the shape of the trichomes covering the tergites. Compared with the most closely related species *Propolyxenus lawrencei* Condé, 1949, from Natal (Champagne Castle, Drakensberg Mountains, alt. 6000 ft.), *P. squamatus* sp. n. shares the following characters: 6 ocelli; internal trichobothrium shorter than the other 2; number and shape of sensilla on antennal articles VI and VII (Condé 1949, p. 125–126, 1959); surface of the labrum with numerous papillae, the 2 or 3 anterior rows larger.

The new species shows the following important differences from *P. lawrencei*:

Position and structure of trichomes on tergites: on each tergite (except collum and telson) the trichomes are arranged in 3 rows and 2 lateral tufts; each paired tuft being connected by posterior and anterior rows of trichomes; the middle row has more spaced trichomes. In *P. lawrencei*, the trichomes are arranged in 3 or 4 irregular rows, forming 2 elongated lateral areas, slightly separated by a narrow medial space.

There are two types of trichomes: the trichomes of the three rows are wider and flatter than the trichomes of the lateral tufts, pleurites and head, and their shape and structure are different, being observed in the family Polyxenidae for the first time. They can be compared to the scale–shaped trichomes of Synxenidae: the trichomes of the rows are all directed caudally and cover the posterior half of tergites II–X and their internal structure is reinforced by differently distributed chitinous elements (Figs 5C–E). The lateral trichomes are longer and arranged in lateral tufts. It is remarkable that the barbate trichomes of *P. squamatus* sp. n. show a progressive transformation into scale–shaped trichomes in the posterior row of the tergite, representing a transition between the two types of trichomes as if, during the course of evolution, the former trichomes had changed into scale–shaped trichomes. These scale–shaped trichomes are thought to protect the animals from desiccation, abundant rain or other environmental disturbances.

In a key of the genus *Propolyxenus*, *P. squamatus* sp. n. would be easily distinguished from all other congeners as is only species with scale-like trichomes. The other species of *Propolyxenus* are more difficult to identify using morphological characters such as the number of ornamental trichomes or coxal glands of the males. For instance, both *P. patagonicus* (Silvestri, 1903) and *P. australis* Short and Huynh, 2010, bear four pairs of coxal glands (cf. Condé et Massoud 1974, p. 227 for *P. patagonicus*) contrary to the first tentative key proposed by Short and Huynh (2010 p. 15). There is a difficulty in

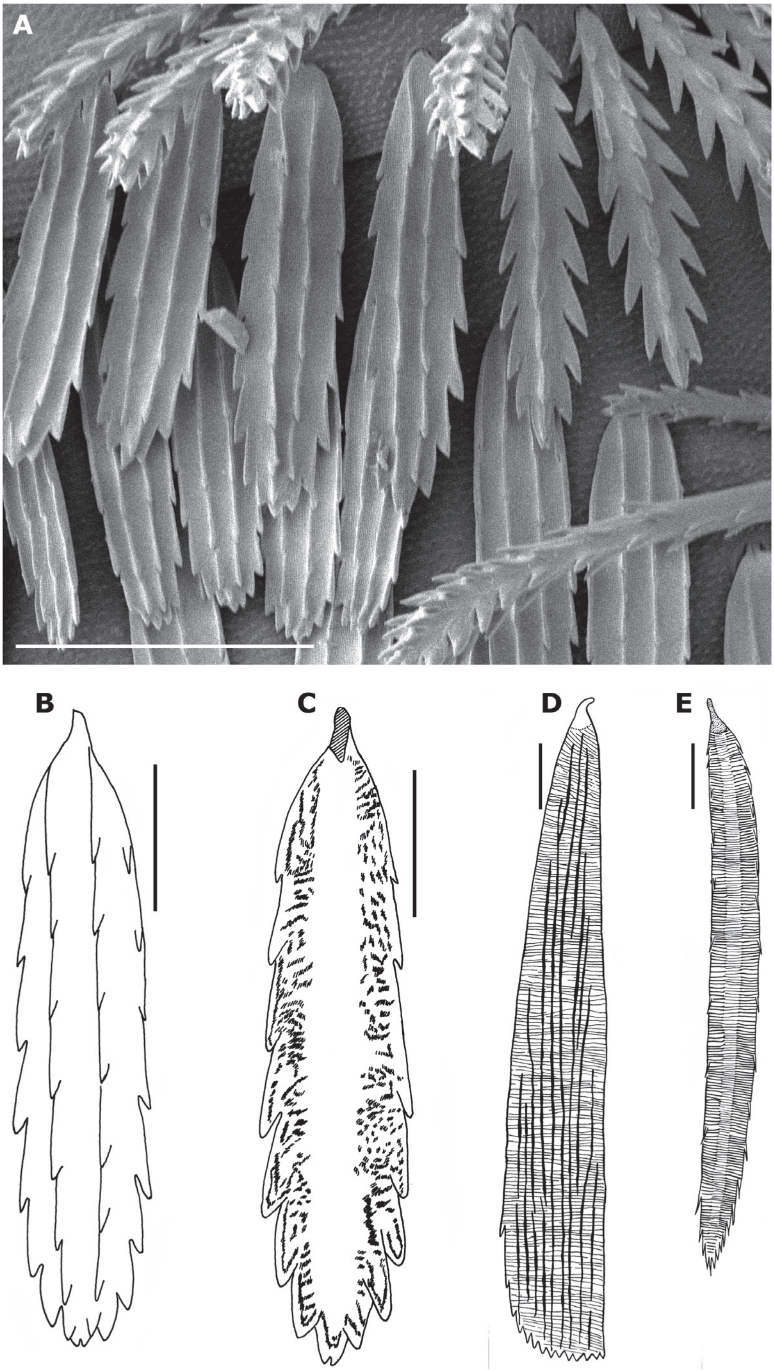


Figure 5. *Propolyxenus squamatus* sp. n. **A** tergal scales **B, C** scale of tergite VII of paratype female. external and internal views respectively **D** scale of tergite VIII of *Phryssonotus capensis* male with 12 pl from Mtuzini, Natal **E** scale of posterior row of tergite VIII of the holotype *Condexenus biramipalpus.* **D** and **E** modified after Nguyen Duy–Jacquemin 2006. Scales bars: **A** 50 µm **B–E** 25 µm.

the limited nature of keys based (even partly) on characters such as coxal glands, requiring collection of adult males. More appropriate characters need to be determined for a more robust key for the genus.

Superfamily Synxenoidea Silvestri, 1923
Family Synxenidae Silvestri, 1923

Genus *Phryssonotus* Scudder, 1885

Synxenus Silvestri, 1900
Kubanus Attems, 1926
Koubanus Attems, 1928
Schindalmonotus Attems, 1926
Lophonotus Menge, 1854, preoccupied, non Stephens, 1829
Kaubanus (sic) Attems, 1929, misprint by Jones 1937
Schindelmonatus (sic) Attems, 1929, misprint by Jones 1937

The genus *Phryssonotus* is characterised by the tergites having dark striated, scale-shaped trichomes directed caudally, all the others being long, dark barbate trichomes; trichomes A and B on head close to trichobothria, one of them being shorter and different from the two others.

Phryssonotus brevicapensis **Nguyen Duy–Jacquemin, Uys & Geoffroy, sp. n.**

urn:lsid:zoobank.org:act:DADD9CDA-BB36-491F-85BB-FE609759F2E9
http://species-id.net/wiki/Phryssonotus_brevicapensis
Figs 6–9

Type material. South Africa, Cape Town,Table Mountain National Park. Tokai S, site 30, Fynbos, altitude 310 m, 34°04'01S, 18°24'03E, leaf litter, 24/XI/2008, holotype adult male (no. 16a) and 1 paratype adult male (no. 16b) (MNHN). Other paratypes: adult male (no. 1) (MNHN), Newlands, site 4, pine plantation, altitude 260 m, 33°58'24S, 18°26'27E, sugar–baited ant trap, 15/I/2009; adult female (no. 20) (MNHN), Tokai S, site 31, pine plantation, altitude 300 m, 34°03'54S, 18°24'10E, decaying log, 19/I/2009; adult female (no. 11) (MNHN), Constantia Nek, site 19, felled pine, altitude 330 m, 34°00'20S, 18°24'45E, pitfall trap, 02/II/2009; male with 14 pl (subadult) (no. 8) (MNHN), Cecilia, Spilhaus, site 16, felled pine, altitude 470 m, 34°00'04S, 18°24'46E, pitfall trap, 23/0I/2009; female with 14 pl (subadult) (no. 13) (MNHN) and female with 12 pl (no. 15) (MNHN), Tokai N, site 27, pine plantation, altitude 330 m, 34°02'23S, 18°23'53E, leaf litter, 21/XI/2008; 2 larvae with 10 pl and 8 pl (no. 12) (MNHN), Orange Kloof, site 22, pine plantation, altitude 240 m, 34°00'23S, 18°24'02E, leaf litter, 18/XI/2008. All specimens collected by Charmaine Uys.

Figure 6. *Phryssonotus brevicapensis* sp. n, habitus, dorsal view, body length: 5 mm. (Photograph by M. Judson).

1 male with 14 pl (no. 6b) was collected at the same site as a male with 12 pl of *Propolyxenus squamatus* sp. n. (no. 6, used for SEM).

Etymology. Refers to the shorter body length and development compared to the most closely related species, *P. capensis*.

Diagnosis. 10 ocelli; 5 trichomes B close to the smallest trichobothrium (tc) as in *P. capensis*. Differing from *P. capensis* with 10 rings in adults instead of 11 (without telson), 15 leg pairs instead of 17, and the attendant shorter post–embryonic development. Males with 3 pairs of coxal glands on legs 7–9.

Description of adults, males and females. Measurements. Body length (without caudal penicil): 4.00–4.50 mm; caudal penicil length: 0.90–1.00 mm (Figs 6, 7A).

Head with 10 ocelli on each side (Fig. 8A); 3 trichobothria with the anterior 1 (tc) possessing a much shorter sensory hair than the other 2 (ta and tb). 5 short frontal trichomes B1–B5 and 1 long, curving trichome A (Fig. 8B).

Proportions of antennal articles as in Fig. 8C. Antennal article VI with 3 basiconic sensilla (Fig. 8C, E): 2 anterior (a), which are shorter and slightly thinner than the posterior one (p); 1 anterior setiform sensillum (s) and 1 posterior coeloconic sensillum (c); antennal article VII (Fig. 8D) with 2 basiconic sensilla, the anterior (a) slightly shorter than the posterior one (p), 1 setifom sensillum (s) between the 2 basiconic sensilla and 1 posterior coeloconic sensillum (c).

Surface of labrum with numerous, small, short cuspidate papillae; papillae of anterior 3 or 4 rows larger, the size of the following papillae decreasing progressively, the smaller ones in the posterior third; about 30 lamellate teeth at anterior margin. Clypeo–labrum with ca. 10-setae, about 3/4 maximum width of labrum. Lateral expansions of gnathochilarium about twice as long as diameter of middle palp, with 21–25 sensilla, middle palp with 26–29 sensilla, of which antero–medial sensilla shorter than the others (Fig. 8F).

Trunk of adults with 11 tergites (including collum and telson) and 15 pairs of legs (Fig. 7A). Collum with 2 medial, separate oval clusters comprising 80–90 barbate

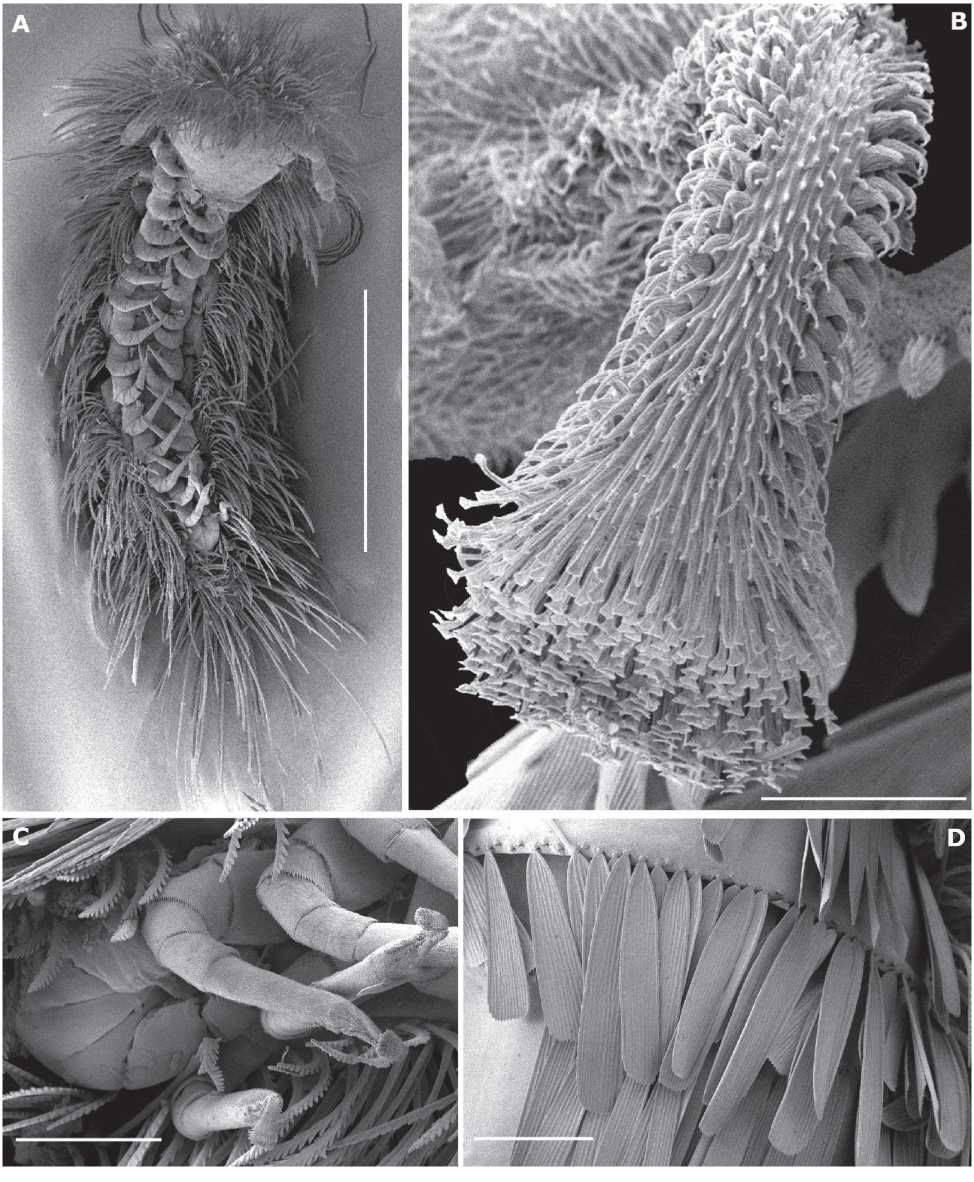


Figure 7. *Phryssonotus brevicapensis* sp. n. **A** habitus, adult male, ventral view showing 15 pl **B** detail of a palette of left leg 15 **C** postero–ventral view showing leg pairs 14 and 15 terminating in palettes **D** detail of scales arranged along posterior margin of tergite. Scale bars: **A** 1 mm **B** 10 µm **C, D** 100 µm.

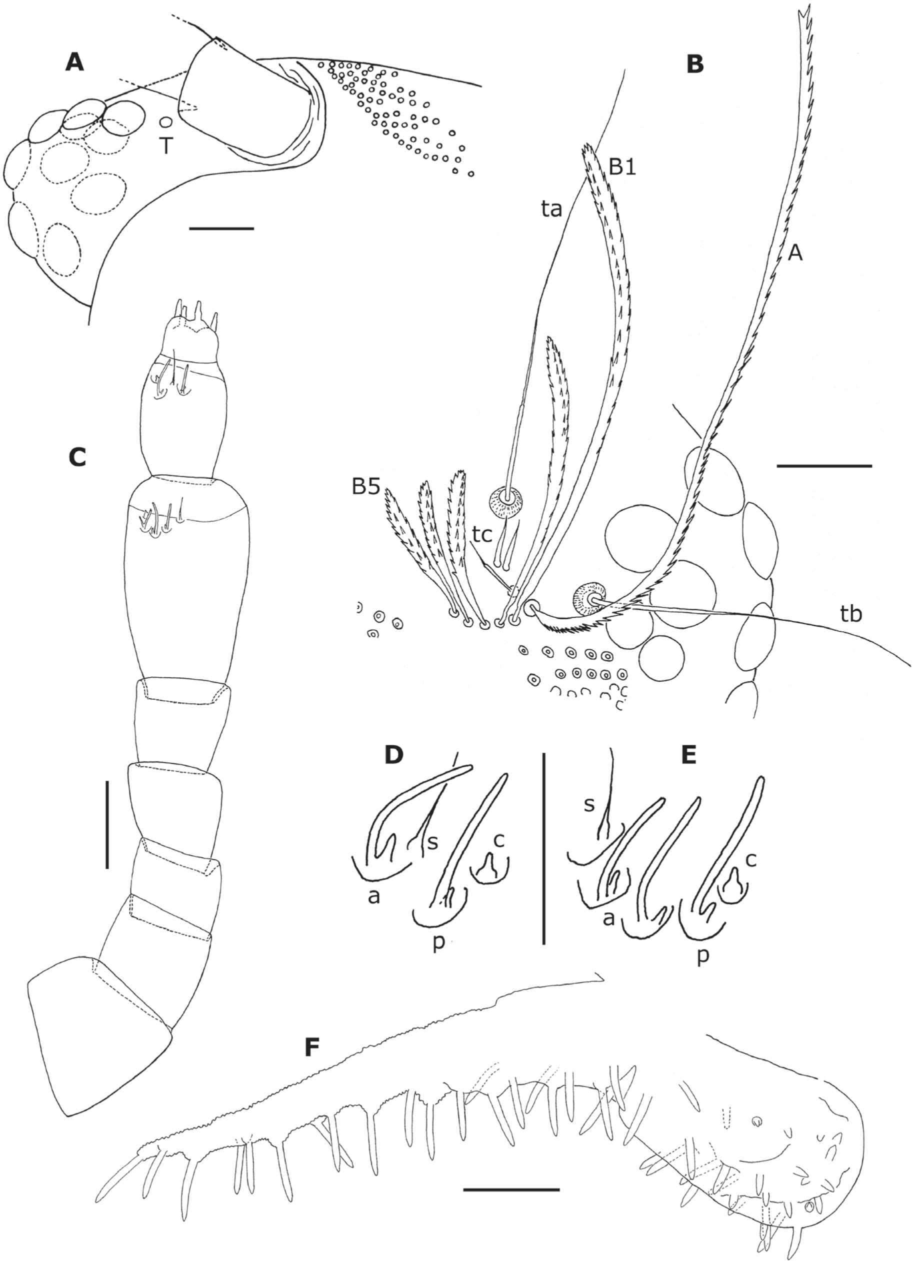


Figure 8. *Phryssonotus brevicapensis* sp. n. **A** right ventral part of head of female adult (no. 20) showing the 10 ocelli, dorsal ones shown with dotted lines **B** right part of the head of holotype showing position of trichobothria ta, tb and tc, long frontal trichome A and short trichomes B1–B5 (only some ocelli drawn) **C** left antenna of holotype; the posterior sensillum is abnormally bifurcated on article VI **D, E** antennal sensilla on articles VII and VI of right antenna of holotype **F** right palp of gnathochilarium of holotype. Abbreviations: **a** anterior basiconic sensillum **c** coeloconic sensillum **p** posterior basiconic sensillum **s** setiform sensillum; T, Tömösvary's organ. Scale bars: **A, B, C** 50 µm; others, 25 µm.

trichomes and a lateral group of 8–14 barbate trichomes. Tergites II–X with submedial and posterior rows of scale–shaped trichomes directed caudally (Fig. 7D), the posterior row arranged along the posterior margin of the tergite; 1 area of aligned barbate trichomes at end of each row, except on tergite II where are 35–40 barbate trichomes arranged, on each side, in 2 diagonal lines above the first scale–row; 2 short rows of barbate trichomes at end of submedial scale–row and 4 (sometimes 5 on tergite II and X) short rows of barbate trichomes at end of posterior scale–row. The number of scales by row ranges from 29–62 on tergites II–X.

Legs short (Fig. 9A), with 8 articles except on legs 1, 14 and 15; last 2 pairs (14–15) without telotarsus, tarsus II terminated in palettes (Figs 7B, C; 9E, F); palettes covered by numerous cuticular setae of different types (Figs 7B; 9F); apodeme of palettes (pa) and claws (ca) extending into distal part of trochanter and linked to the flexor unguiculi muscle, which allows a great flexibility of the palette of the leg pairs 14 and 15, as well as to the claws of legs 1–13, in accordance with the description given by Manton (1956) for *Polyxenus lagurus*. Legs 1–13 with each trochanter, prefemur (Fig. 9B), femur, tibia and tarsus I bearing a single long and very fine seta; seta of second tarsus longer than claw (Fig. 9C). Legs 14 and 15 with only 2 setae on prefemur (Fig. 9E). Telotarsus bearing an anterior process (ap) with spinous projection longer than claw; lamellate process (plp) thickened and basally pleated; claw with 2 subequal, strongly pointed latero–anterior and posterior teeth (Fig. 9C, D).

Female: large vulval sacs elongated, reaching as far as fourth pair of legs and bearing numerous small setae inserted in parallel circles and sparse longer setae.

Male: all areas of penis with usual thin cuticular setae and about 15 longer setae (holotype). Coxal glands on legs 7–9.

Conical telson with a transverse row of 15 (male no. 1), 10 (male no. 16b) or 14 (female no. 20) scale–shaped trichomes with each lateral end prolonged by barbate trichomes; long barbate trichomes on distal part.

Subadults. 1 male, 1 female: Measurements: Body length (without caudal penicil): 3.20 mm (male no. 8) and 3.90 mm (female no. 13); caudal penicil length: 0.90 mm. 14 pairs of legs, the 14th terminating in a palette; 1 pair of appendage–buds on lateral side of anal valves, from which 15th pair of legs will develop, the future adult stadia having the leg pair 15 terminating in palettes. Other characters as in adults, except no scale–row on telson.

Male: Coxal glands on legs 7–9.

Female: large vulval sacs elongated, reaching as far as fourth pair of legs.

Stadium VII. 1 female (no. 15) with 12 pl; body length (without caudal penicil): 3.80 mm; caudal penicil length: 0.80 mm; 10 ocelli, vulval sacs elongated, reaching as far as third pair of legs. 2 pairs of external buds.

Stadium VI. 1 juvenile male with 10 pl; body length (without caudal penicil): 3.30 mm; 10 ocelli; rudimentary coxal glands on legs 7 and 8. 2 pairs of external buds.

Stadium V. 1 larva with 8 pl; body length (without caudal penicil): 2.70 mm; 9 ocelli. 2 pairs of external buds.

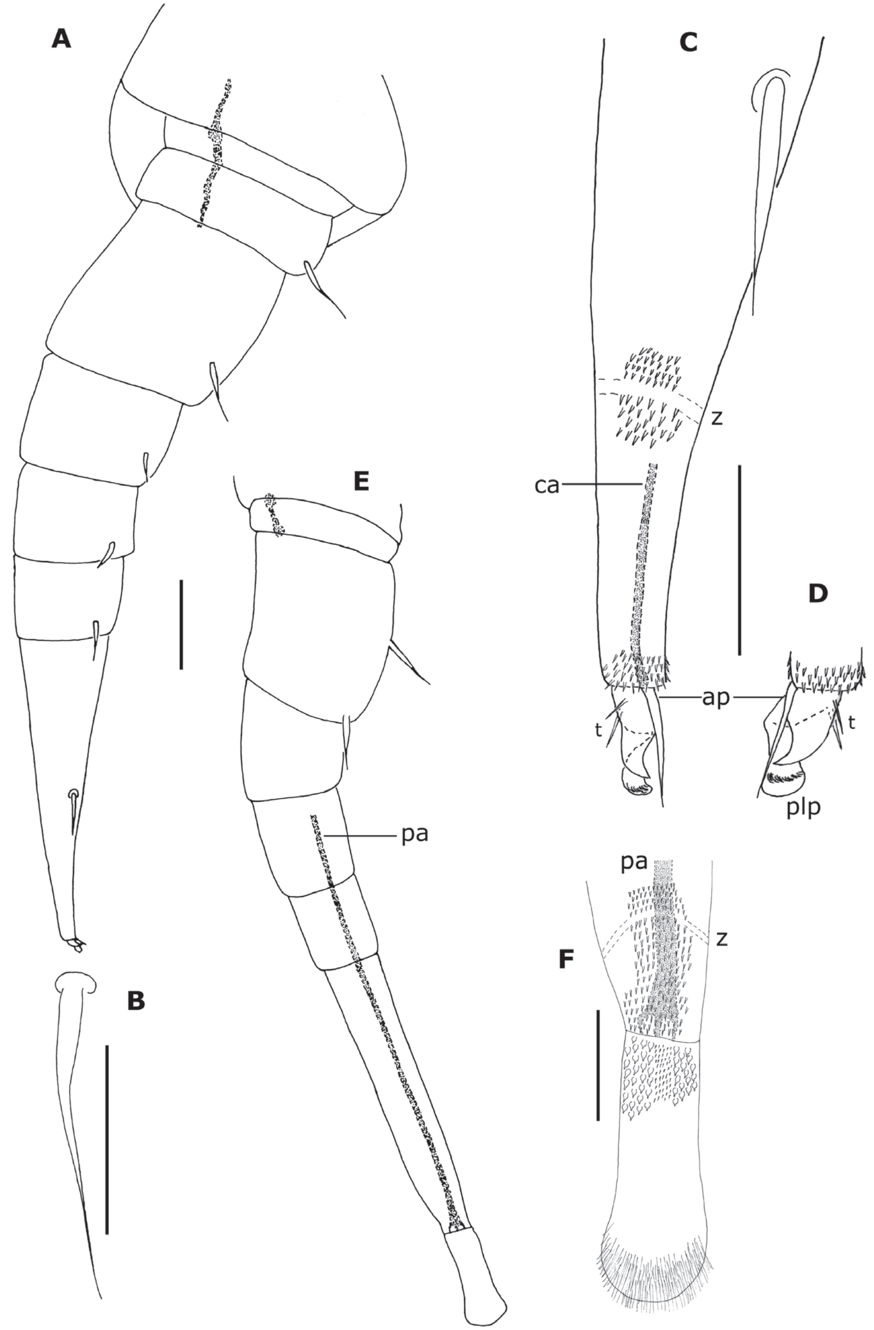
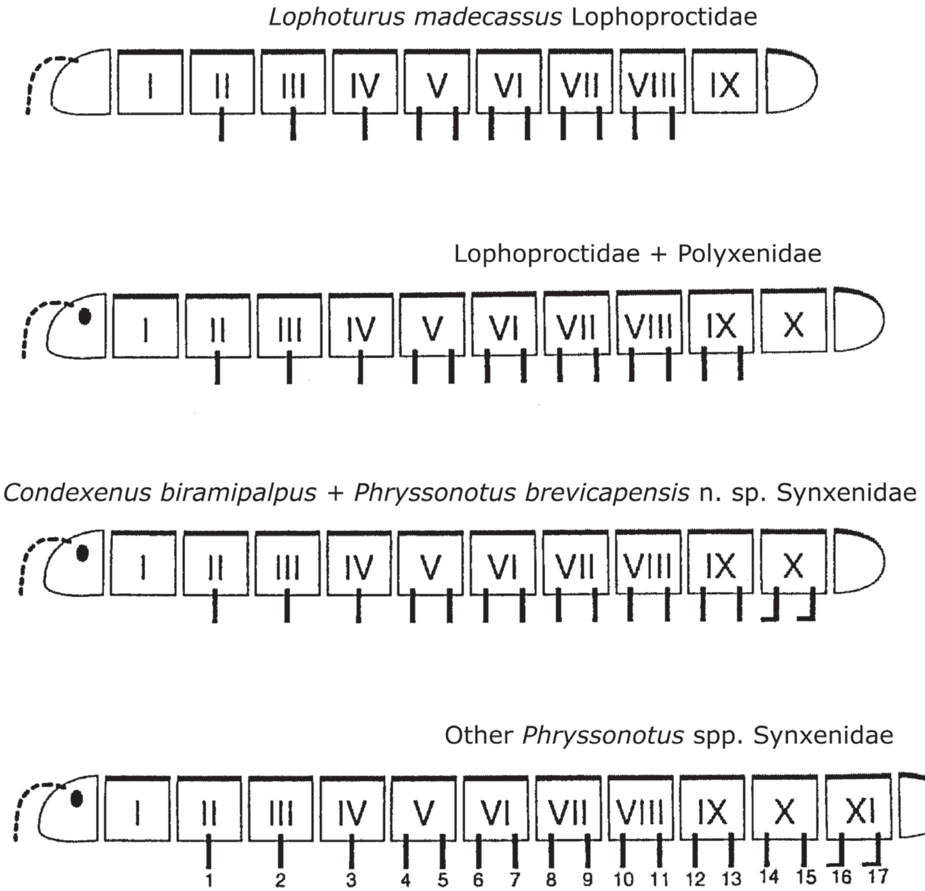


Figure 9. *Phryssonotus brevicapensis* sp. n., adult male (no. 1). **A** right leg 12 **B** seta of prefemur of right leg 12 **C** distal part of tarsus II of the right leg 12 **D** telotarsus of left leg 12 **E** right leg 15 **F** distal part of tarsus II and palette of right leg 15. Abbreviations: **ap** anterior process **ca** apodeme of claw **pa** apodeme of palette **plp** posterior lamellate process **t** latero–anterior and posterior teeth **z** smooth area. Scale bars: **A, E** 50 µm; others, 25 µm.

Discussion. *Phryssonotus brevicapensis* sp. n. exhibits all the general characters usually present in the family Synxenidae: long and thin dark barbate trichomes all along the body, tergites covered by tergal scale–shaped trichomes that are striated and arranged in 2 transverse rows along all the tergites except the collum; telson subconical; elongated vulvae; and last 2 leg pairs terminating in palettes instead of claws. It also shows the typical structure of the scale–shaped trichomes found in the genus *Phryssonotus*. *Phryssonotus brevicapensis* sp. n. differs from other members of the genus in having 11 tergites and 15 pl; the last 2 pairs (14th and 15th) terminating in palettes; and males with 3 pairs of coxal glands on legs 7–9. These differences are strongly related to biology and development, and justify the creation of a new species. All other species of *Phryssonotus* have 12 tergites and 17 pairs of legs with the last 2 pairs (16th and 17th) terminating in palettes, and males with 3 pairs of coxal glands on legs 9–11. Due to its shorter length and the position of the coxal glands on legs 7–9, it is similar to *Condexenus biramipalpus*. The elongated vulvae (ovipositors) of the females also resemble those


Lophoturus madecassus Lophoproctidae

Lophoproctidae + Polyxenidae

Condexenus biramipalpus + *Phryssonotus brevicapensis* n. sp. Synxenidae

Other *Phryssonotus* spp. Synxenidae

Figure 10. Comparison of segmentation in Polyxenida, corrected and improved after Nguyen Duy–Jacquemin (2006).

of *Condexenus* species in reaching as far as the fourth pair of legs, as opposed to sixth at most in other *Phryssonotus* species.

P. brevicapensis sp. n. is most closely related to *P. capensis*, in having 10 ocelli and 5 short frontal trichomes B1 to B5, *P. capensis* has 5–6 trichomes B (Silvestri 1923).

Following the discovery of *Condexenus biramipalpus* from Namibia, it is of great interest to add *Phryssonotus brevicapensis* sp. n. as the second example of reduction of ring and leg number in the family Synxenidae, whose representatives bear the largest number of segments among the Penicillata. This supports the hypothesis of a trend towards a shortened postembryonic development during the course of evolution of Polyxenida (Condé 1969, Nguyen Duy–Jacquemin 2006). The comparison of the pattern of development is emphasised in the improved scheme (Fig. 10), in which the shortest development pattern is seen in *Lophoturus madecassus* (Marquet et Condé, 1950). The two new species described in the present work strongly support this evolutionary trend among penicillate families.

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The genus *Unixenus* Jones, 1944 (Diplopoda, Penicillata, Polyxenida) in Australia

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Abstract

The penicillate genus *Unixenus* Jones, 1944 is widespread, with species found in Africa, Madagascar, India and Australia. Each of the two Australian species was originally described from single samples from Western Australia. In this study, collections of Penicillata from museums in all states of Australia were examined to provide further details of the two described species, to revise the diagnoses for both the genus and the species, and to better understand the distribution of the two species in Australia. In addition, two new species *Unixenus karajinensis* **sp. n.** and *Unixenus corticolus* **sp. n.** are described.

Keywords

Diplopoda, Penicillata, Polyxenidae, new species, Australia, distribution, taxonomy

Introduction

Jones (1937) created the genus *Monoxenus* for his type species *Monoxenus padmanabhii* collected from the Trivandrum region in Southern India, later renaming it *Unixenus* Jones, 1944, because *Monoxenus* had been preoccupied by a genus in Cerambycidae (Coleoptera). Jones distinguished *Unixenus* from the genus *Monographis* Attems, 1907 on the grounds that the new genus had shorter tergal setae and caudal trichomes with one to five hooks rather than two hooks. However, these characters can vary within a genus, for example *Propolyxenus* Silvestri, 1948 (Short and Huynh 2010). Silvestri (1948) separated *Monographis* from *Unixenus* on the basis of the absence of the tarsal spine and this absence was then used (Nguyen Duy-Jacquemin and Condé 1967) to create the two Australian species *Unixenus attemsi* Nguyen Duy-Jacquemin and Condé, 1967, based on specimens originally identified by Attems (1911) as the African *Monographis schultzei* Attems, 1909, and *Unixenus mjoebergi* (Verhoeff, 1924) originally placed in *Monographis*. Nguyen Duy-Jacquemin and Condé (1967) also reassigned two species formerly placed in the genus *Saroxenus* Cook, 1896 to the genus *Unixenus*, namely *Saroxenus broelemanni* Condé & Jacquemin, 1962 from Madagascar and *Saroxenus vuillaumei* Condé & Terver, 1963 from the Ivory Coast. In this paper, additional characteristics of the two species *Unixenus mjoebergi* and *U. attemsi* are described based on examination of numerous museum specimens and fresh material collected by the authors. Two new species from Australia are also described.

Materials and methods

Some specimens for this study were obtained by sieving samples of bark and decomposing leaf litter into a white tray and hand-picking into 70% ethanol. The majority of specimens in this study, however, came from Australian museum collections. Specimens were examined using light and scanning electron microscopy. For light microscopy, specimens were cleared in 15% potassium hydroxide, heated in a water-bath for 2 minutes at 80°C, neutralised in 20% acetic acid for 2 minutes, rinsed in distilled water and dehydrated in a series of ethanol baths prior to staining with 1% Fast Green solution to increase contrast. The head and body were separated, the body cut open with a single latero-longitudinal incision and contents removed. After rinsing in 100% ethanol, stained specimens were transferred to 100% isopropanol, then to xylene and mounted on slides with DPX synthetic resin. Scanning electron micrographs were obtained for adults of *U. corticolus* sp. n., and adults and one subadult stadium VII of *U. karajinensis* sp. n. The specimens were preserved in 70% ethanol prior to being gently mounted on stubs using adhesive tabs, then air-dried, sputter-coated with gold and examined with a Philips XL20 scanning electron microscope.

Specimen lengths were measured from head to telson with caudal bundle of trichomes excluded. Adults were sexed when possible. Measurements are an indication only of size as length varies with state of activity in life and state of preservation in death. Naming of the leg segments follows Manton (1956). Unless otherwise indicated, all millipedes referred to are adults (stadium VIII). Stadium VII specimens are referred to as subadult, and "immature" refers to any non-adult stadium. The trichomes in a transverse row on the telson dorsal to the caudal bundle are referred to as ornamental trichomes.

Abbreviations: AM = Australian Museum, Sydney, New South Wales; ANIC = Australian National Insect Collection, Canberra, Australian Capital Territory; MV = Museum Victoria, Melbourne, Victoria; NSW = New South Wales; Qld = Queensland; QM = Queensland Museum, Brisbane, Qld; SA = South Australia; Tas = Tasmania; Vic = Victoria; WA = Western Australia; WAM = Western Australian Museum; L = left; R = right.

Results

Subclass Penicillata Latreille, 1831
Order Polyxenida Verhoeff, 1934
Superfamily Polyxenoidea Lucas, 1840
Family Polyxenidae Lucas, 1840
Genus *Unixenus* Jones, 1944

Unixenus Jones, 1944

http://species-id.net/wiki/Unixenus

Monoxenus Jones, 1937: 138; Silvestri 1948: 216.
Unixenus Jones, 1944: 94; Nguyen Duy-Jacquemin and Condé, 1967: 68.

Diagnosis. The genus is typical of the family Polyxenidae with 10 tergites plus telson, 13 pairs of legs and 8 ocelli in each eye. Antennal article VII has 2 thick basiconic sensilla with 1 setiform sensillum between, and 1 coeloconic sensillum posteriorly. Gnathochilarium has long lateral palps at least 1.5 X diameter of medial palp, 20–22 simple sensilla on medial palp and 13 on lateral palp. Spine on tarsus 2 absent, replaced with small setiform sensillum. 2 or more rows of barbate trichomes posteriorly on each tergite with trichomes arranged in 2 broad clusters either side of the midline, with a small gap between clusters. Posterior row of trichomes continuous or with medial gap, anterior row often uneven, intermediate trichomes rarely in defined rows. Telson with single caudal bundle containing trichomes with 1–11 hooks, ornamental trichomes with distinct dark long trichomes *c* either side midline.

Type species. *Unixenus padmanabhii* (Jones, 1937).

Unixenus attemsi Nguyen Duy-Jacquemin & Condé, 1967

http://species-id.net/wiki/Unixenus_attemsi
Figs 1A, 2, 3C, 3D

Unixenus attemsi Nguyen Duy-Jacquemin and Condé, 1967: 68, figs 9, 10.

Material examined. Slide preparations were made of adults from the following localities: Marangaroo Conservation Area in Perth, WA, 31°49’48"S, 115° 50’12"E, 9 February 2006, M. Short and C. Huynh, in *Eucalyptus* bark; Scott Creek, SA, 35°04'37"S, 138°42'29"E, 15 February 2005, M. Short and C. Huynh, litter under *Eucalyptus*;

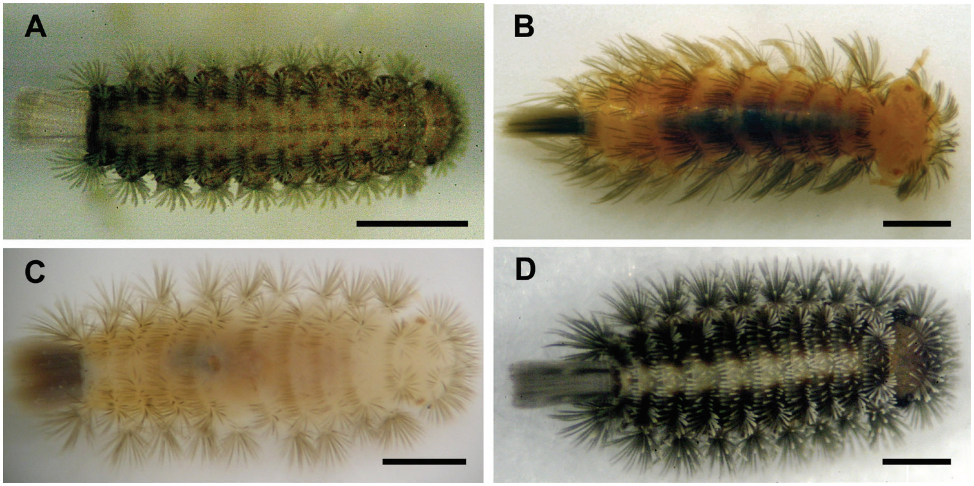

Figure 1. A *Unixenus attemsi* Nguyen Duy-Jacquemin & Condé, 1967, Scott Creek, live adult **B** *U. mjoebergi* (Verhoeff, 1924), Barrow Island, WAM T71082 **C** *U. karajinensis* sp. n., Wittenoom, WAM T71106 **D** *U. corticolus*, sp. n., Tidal River, live adult. Scale bars = 0.5 mm.

Robinvale, Vic, 34°35'S, 142°46'E, 28 October –3 November 1968, T. Weir, J. Lawrence and E. Hansen, litter under *Eucalyptus camaldulensis,* ANIC berlesate 1085. Collections made by the authors will be deposited in WAM and MV.

Diagnosis. This species can be distinguished from other species in the genus by the presence of 2 transverse rows only of short barbate trichomes on collum and tergites, 3 basiconic sensilla on antennal article VI, long lateral palps on gnathochilarium (2.5 X diameter of medial palp), 1 seta on femur and no setae on tibia, funicle of leg setae with no projecting spines, telotarsus with 4–6 processes on claw, thin anterior spinous projection same length as claw, 3 ornamental trichomes *c* each side, caudal hooked trichomes with 3–11 hooks with double pointed barbs on stem of trichomes with 4 or more hooks.

Remarks. The original description by Attems (1911) was very brief. A redescription of the species by Nguyen Duy-Jacquemin and Condé (1967) was based on examination of two female specimens from Torbay in southern Western Australia (the type locality) held in the Zoology Museum, Hamburg, Germany (coll. by Hamburg S.W. Australia Expedition 1905). This description is detailed and clearly illustrated, but in light of recent collection of the species from eastern Australia and examination of large numbers of the species from a range of locations in WA, the species description can now be expanded and variation in some characters recorded.

Additional description. Body unpigmented with exception of darkly pigmented medial longitudinal band on dorsal surface and darkly pigmented lateral projections. Trichomes including caudal bundle colourless (Fig. 1A). Body length from Robinvale and Scott Creek specimens 1.2–1.7 mm (n = 6), Perth 1.4–1.8 mm (n = 5), Walpole-Nornalup National Park, WA, WAM T71144, 2.3 mm; caudal bundle 0.3–0.4 mm. Variation noted in number of trichomes in rows on posterior vertex of head, between specimens and between left and right (as determined by examination of trichome inser-

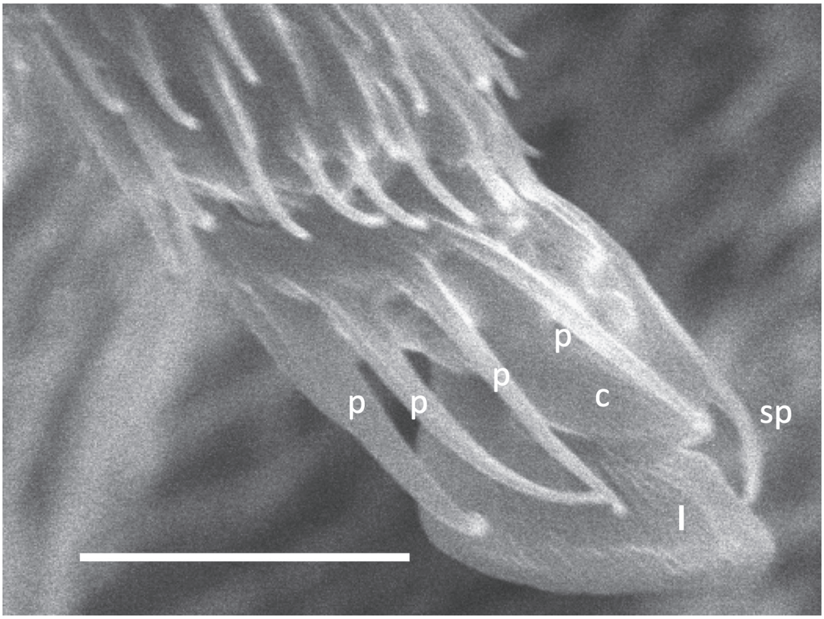

Figure 2. *Unixenus attemsi* Nguyen Duy-Jacquemin & Condé, 1967, adult male, Perth. Telotarsus showing 4 processes attached to the base of the claw. p = process, c = claw, sp = anterior spinous projection, l = lamella process. Scale bar = 5 µm.

tion points). Specimens examined varied with numbers each side: Perth 16–19 (n = 5), Scott Creek 12–13 (n = 4), Robinvale 9–14 (n = 7), no difference between males and females, individual maximum difference between left and right sides of vertex = 2. Clypeo-labrum as previously described, with 8 setae most commonly observed along posterior margin, in contrast to 9 described by Nguyen Duy-Jacquemin and Condé (1967).

Collum shows variation in trichome number: Perth 19–25 each side (n = 4), Scott Creek 16–19 (n = 2), Robinvale 15–16 (n = 1). Maximum variation between right and left sides on a single individual = 2. On tergite 2, trichome numbers varied (each side of midline): Perth 23–32 (n = 4), Scott Creek 19–23 (n = 4), Robinvale 20 (n = 1). Males with 2 pairs of coxal glands, leg pairs 8 and 9. Variation observed in number of ornamental trichomes *a*: Perth 7–10*a* (each side) (n = 5), Scott Creek 6–7*a* (n = 5), Robinvale males 4–5*a* (n = 2), females 6–9*a* (n = 2). Adult females described by Nguyen Duy-Jacquemin and Condé (1967) had 10 and 12 trichomes *a* each side. Circular indentation (labelled *d*) observed adjacent and external to each cluster trichomes *c* (Fig. 3C). This structure, also illustrated in Figure 3G in Condé and Jacquemin (1962) labelled '*x*', is present in all adult specimens of the genus examined but function unknown. Telotarsus with 4 processes on claw rather than 6 processes previously observed (Nguyen Duy-Jacquemin and Condé 1967) (Fig. 2).

Single caudal bundle of hooked trichomes, 2–11 hooks with barbed stems, size of hook and nature of barbed stem dependent on number of hooks: trichomes with 2–4 hooks, hooks large, all barbs on stem distal-facing similar to those in *U. mjoebergi*

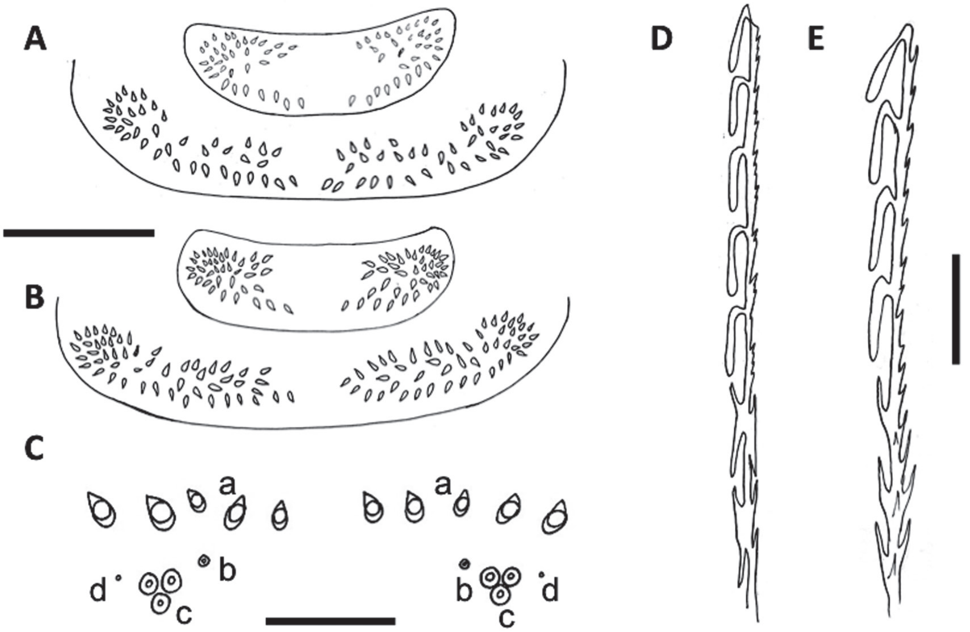

Figure 3. A Pattern of trichome insertions in collum and tergite 2 of *U. mjoebergi* (Verhoeff, 1924) from Eil Eil Spring, WAM T71082 **B** Pattern of trichome insertions on collum and tergite 2 of *U. mjoebergi* from Hann Tableland, QM S17167 **C** Pattern of ornamental trichome insertions *a, b, c* and circular indentations *d, U. attemsi* Nguyen Duy-Jacquemin & Condé, 1967, adult female, Robinvale, ANIC berlesate 1085 **D** Hooked trichome with double barbs on stem, *U. attemsi,* adult female, Robinvale, ANIC berlesate 1085 **E** Hooked trichome with single barbs on stem, *U. mjoebergi,* Barrow Island, WAM T71112. Scale bars: A & B (shared) = 200 µm; C = 30 µm, D & E = 40 µm.

(Fig. 3E), trichomes with 4–11 hooks, hooks smaller and with double barbs along stem proximally, with both proximal- and distal-facing points (Fig. 3D). Specimens examined by Nguyen Duy-Jacquemin and Condé (1967) had 5–7 hooks, with no description given of barbs along stem.

Distribution. This species is widespread in Australia (Fig. 8) but appears to be most common in a range of habitats in southern WA. The most northerly collection is Bush Bay, WA, 25°07’03"S, 113°48’22"E, where it was collected together with *U. mjoebergi* (WAM T71125). In eastern Australia the species appears to be restricted to dry woodlands. It is found under bark of *Eucalyptus* in small aggregations, often with many exuviae, as well as in dry leaf litter and under stones in treed areas on well-drained sandy or sandy loam soil.

Remarks. The two Torbay specimens described by Nguyen Duy-Jacquemin and Condé (1967) are longer than almost all measured in this study, at 2.25 and 2.65 mm. Torbay is on the south coast of WA (Fig. 8) and a single specimen in the WAM collection (T71144) from Walpole-Nornalup National Park on the south coast of WA is also large (2.3 mm). All adults from eastern Australia are smaller. Smaller adults have fewer trichomes on head and vertex.

Unixenus mjoebergi **(Verhoeff, 1924)**

http://species-id.net/wiki/Unixenus_mjoebergi
Figs 1B, 3A, 3B, 3E

Monographis mjoebergi Verhoeff, 1924: 38.
Unixenus mjoebergi Nguyen Duy-Jacquemin and Condé, 1967: 73, figs 11, 12.

Material examined. Slide preparations were made of adults from the following collections: Barrow Island, WA, 20°47'38"S, 115°27'24"E, sites 17 (2 suction samples) and 105 (2 samples: suction and Winkler), 24 April 2005, K. Edward and S. Callan, WAM T71111–4; Eil Eil Spring, WA, 19 °47'S, 121°26'E, 20 September 2002, K. Edward, in litter, WAM T71082; Hann Tableland, Qld, 16°49'S, 145°11'E, 950 m, 12 December 1995, G. Monteith, pyrethrum spray on bark, open forest, QM S38765; islands in Capricornia Cays National Park, Qld, pitfall trapping by QM and Queensland Parks and Wildlife Service: Masthead Island site 1, 23°32'24"S, 151°43'44"E, 0–5 m, 5–7 October 2008, grassed areas on beach, QM S17167; Erskine Island site 2, 23°30'07"S, 151°46'23"E, 2 m, 6–8 October 2008, open grassland, QM S17561; Lady Elliot Island site 7, 24°06'50"S, 152°42'58"E, 0–5 m, 29–31 March 2008, beach bean vine thicket, QM S16031; West Hoskyn Island site 1, 23°48'32"S, 152°17'20"E, 0–5 m, 13–15 May 2008, *Casuarina* stand, QM S17052; One Tree Island site 1, 23°30'25"S, 152°05'31"E, 0–5 m, 23–25 September 2008, *Casuarina* stand, QM S17144; North West Island site 8, 23°17'31"S, 151°42'29"E, 0–5 m, 9–11 October 2008, *Casuarina* stand, QM S17609; and Eungella, 21°12'S, 148°26'E, 600 m, 18 November 1981, Gillison, ANIC berlesate 984.

Diagnosis. This species can be distinguished from other species in the genus by the presence of 3 or more transverse rows of barbate trichomes on collum and tergites 2 and 3, with 2 or more rows in remaining tergites, 3 basiconic sensilla on antennal article VI, long lateral palps on gnathochilarium (2.5 X diameter of medial palp). 2 setae on femur and 1 seta on tibia, funicle of setae of coxa, prefemur and femur ridged, ridges extending as projections surrounding flagellum, telotarsus with 2 processes (anterior and posterior) on claw, anterior spinous projection longer than claw, 3 ornamental trichomes *c* each side, caudal hooked trichomes with 1–4 hooks, absence of double pointed barbs on stem.

Remarks. The original description by Verhoeff (1924) was very brief and no illustrations were given. A redescription of this species by Nguyen Duy-Jacquemin and Condé (1967) was based on a single paratype adult female preserved in ethanol from the Museum of Comparative Zoology, Harvard University. This description is detailed and clearly illustrated, but in light of recent collection of the species from eastern Australia and examination of large numbers of the museum specimens from a range of locations in Western Australia and Queensland, the species distribution can now be extended and variation in some characters recorded.

Additional description. No freshly collected specimens available. All specimens had been preserved in 70% ethanol for at least 18 months prior to examination, with most in ethanol for decades. Body yellow brown in colour with trichomes including caudal bundle pigmented dark brown. Average length adult (mm): Barrow Island 2.1–2.5 (n = 3), Hann Tableland 3.0–3.4 (n = 5), Capricornia Cays 2.0–2.8 (n = 15); caudal bundle 0.4–0.5 mm. No differences observed between sexes. Variation in both pattern and number of trichomes on posterior vertex of head in specimens from different populations and variation within a population between specimens, and between left and right on a single individual. Pattern of 3 oblique rows each side separated medially by broad gap as described for paratype by Nguyen Duy-Jacquemin and Condé (1967) most common, but occasionally 2 rows, and 1 specimen with 5 rows each side. Trichomes barbate and longer than those of *U. attemsi*. Variation in trichome number each side as follows: Barrow Island females 22–26 (n = 2), males 20–22 (n = 2); Eil Eil Spring subadult male 18–19; Eungella subadult male 19–21; Hann Tableland subadult males 19–25 (less in posterior row) (n = 3), adult females 23–32 (n = 5); Capricornia Cays, both males and females 17–23 (n = 12), plus 1 male with 5 rows, 34 each side. Clypeo-labrum as described for paratype by Nguyen Duy-Jacquemin and Condé (1967) for all specimens examined, with 12 setae along posterior margin, except for those from Queensland mainland sites Hann Tableland and Eungella with 10 setae (n = 11); further variation was also noted with a few specimens having 3 rather than 2 lamellar teeth each side along anterior margin. Occasional variation noted in number of sensilla on gnathochilarium with 20–22 sensilla each medial round palp and 12–13 each lateral palp. The majority have 22 sensilla on medial palp in contrast to the 21 in the single specimen described by Nguyen Duy-Jacquemin and Condé (1967).

Collum similar to description in Nguyen Duy-Jacquemin and Condé (1967) with trichomes in broad lateral clusters each side of wide medial gap equal to width of cluster, a posterior row extending from lateral edge of each cluster towards the midline. Barrow Island adult specimens similar to paratype (Nguyen Duy-Jacquemin and Condé 1967) with continuous posterior row of trichomes and trichome insertion at midline. However in all other specimens examined, a median gap was present in the posterior row (Figs 3A and B). Number of trichomes each side median gap varied: Barrow Island 39–49 (n = 3), Eil Eil Spring 32–34 (n = 1, subadult male), Eungella 30–31 (n = 1, subadult male), Hann Tablelands 37–43 (n = 4), Capricornia Cays 35–57 (n = 12). In remaining tergites, only Barrow Island specimens were similar to the description by Nguyen Duy-Jacquemin and Condé (1967) with a continuous posterior row in all but tergite 10. In all other specimens examined, a median gap was present in the posterior row of most if not all tergites. Number of trichomes each side on tergite 2 variable: Barrow Island 46–60 (n = 3); Eil Eil Spring 39 (n = 1, subadult male); Eungella 36 (n = 1, subadult male); Hann Tablelands 48–66 (n = 4); Capricornia Cays 43–70 (n = 12). Tergal trichomes barbate and twice as long as those of *U. attemsi*, trichomes longer in more posterior tergites. Males with coxal glands present on leg pairs 8 and 9.

Variation observed in number of ornamental trichomes *a* as follows (number given per side): Barrow Island 4–6 (n = 4, no differences between sexes); Eil Eil Spring 6 (n = 1, subadult male); Hann Tablelands: adult females 3–8 (n = 5), subadult males

8–12 (n = 3); Eungella 7–8 (n = 1, subadult male); Capricornia Cays 4–9 (n = 13, no differences between sexes). Hooked caudal trichomes with 1–4 hooks on barbed stems, barbs all distal-facing (Fig. 3E).

Distribution. *Unixenus mjoebergi* has been identified in north western and north eastern Australia, including islands off the coast on either side of the continent (Fig. 8). The lack of records for central Australia and Northern Territory is possibly more a reflection of very limited and untargeted collecting effort rather than actual absence of the species from these regions. The species is found in litter of treed habitats (both open and closed forest types), and occasionally in sandy beach and sand dune habitats.

Remarks. The specimens examined showed variation in a number of characters, with those of the single female adult paratype described by Nguyen Duy-Jacquemin and Condé (1967) falling within the range for each. Of particular interest is the variation in pattern of tergal trichomes with only the Barrow Island specimens showing the same pattern as the paratype. All specimens from Queensland, as well as the single specimen from Eil Eil Spring, showed a distinct gap in all or almost all tergites (the only exception were two specimens that lacked a gap in posterior row of tergites 4 and 5). Numbers of trichomes, including the ornamental trichomes *a*, varied also with no distinct geographic pattern discernible. The wide distribution, and presence of the species on islands on both sides of the continent points to the possible movement of the species by birds and or wind.

In the course of this study, collections from the Hamersley Ranges of Western Australia previously identified as *Unixenus mjoebergi* were found to have a number of important differences requiring erection of a new species, *Unixenus karajinensis* sp. n.

Unixenus karajinensis sp. n.

urn:lsid:zoobank.org:act:8D5DDD19-2571-437F-AE9D-74B932AD9ABB
http://species-id.net/wiki/Unixenus_karajinensis
Figs 1C, 4, 5

Holotype. Male, Wittenoom Gorge, asbestos mine, WA, 22°19'S, 118°19'E, 20 May 1977, WD Temperton, WAM T71106, mounted on slide.

Paratypes. Six females same data as holotype, on six slides, WAM T116452–7; four females and three males from Tom Price, WA, 22°41'S, 117°47'E, 727 m, 6 February 1978, CE Chobanoff, females: WAM T116458–61, males: WAM T116462–64, mounted on slides.

Other material. Additional specimens, same data as holotype, WAM T116451; additional specimens, same data as paratypes from Tom Price, WAM T116465; AM collection from Hamersley Ranges, WA, 10 km north from Tom Price turnoff along Nanutarra - Wittenoom Rd, on left side, 22°32'21"S, 117°38'01"E, 25 May–4 June 2004, M. Bulbert et al., pitfall traps, AM KS111219.

Diagnosis. Differs from *Unixenus mjoebergi* in longer and thinner tergal trichomes, 6 pairs of coxal glands in males on leg pairs 6–11, telotarsus with anterior spinous

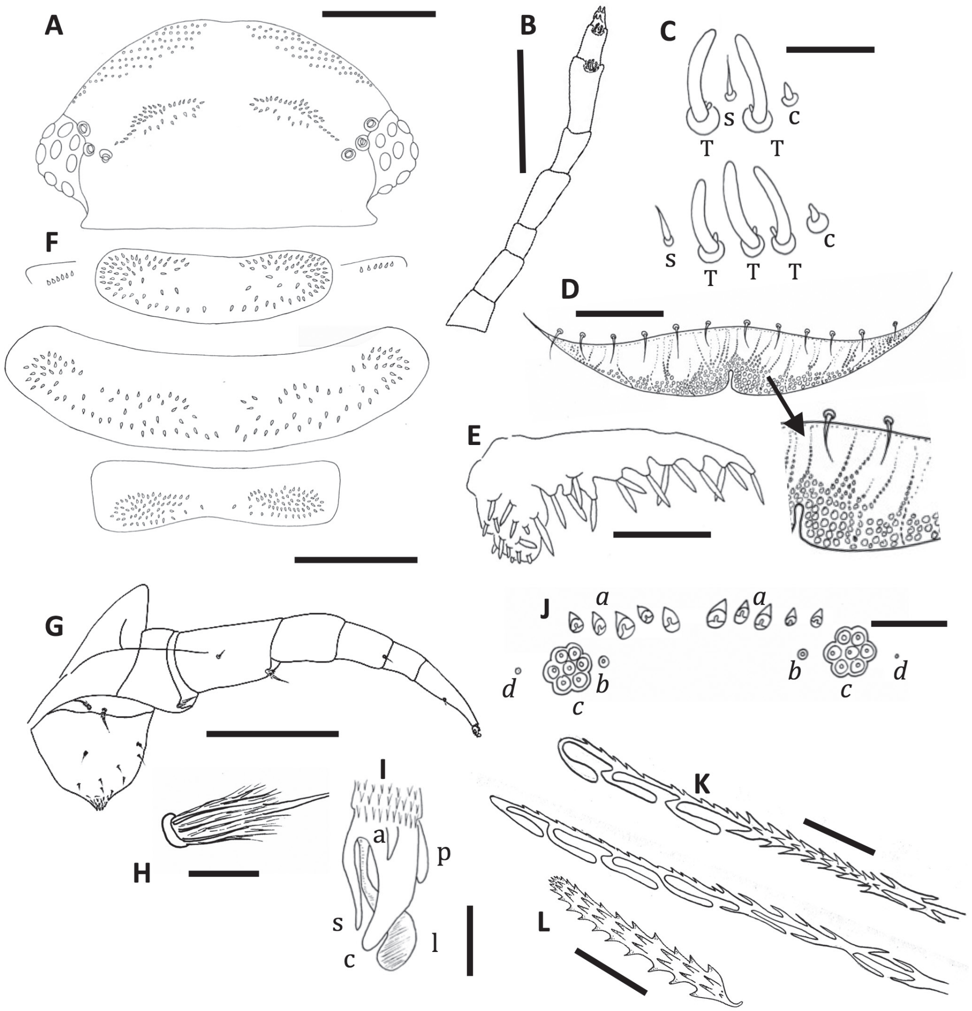

Figure 4. *Unixenus karajinensis* sp. n., holotype, WAM T71106 **A** Head, dorsal view showing arrangement of ocelli, position of trichobothria and trichome insertions **B** Right antenna **C** Details of sensilla on antennal articles VI and VII, sensilla type indicated as follows: coeloconic (c), setiform (s), thick basiconic (T), article VI sensilla lower row **D** Clypeo-labrum with enlargement to show papillae **E** Right palp of gnathochilarium showing long lateral palp, medial palp and simple sensilla **F** Collum, tergite 2 and tergite 10 showing pattern of trichome insertions **G** Left leg 2 showing chaetotaxy on leg segments and penis **H** Details of seta on coxa, prefemur and femur **I** Anterior view of left telotarsus showing anterior spinous projection (s), claw (c) with anterior (a) and posterior (p) processes and lamella (l) **J** Pattern of ornamental trichomes *a, b, c* and circular indentations *d* **K** Distal portion of typical hooked trichomes showing double headed barbs **L** Trichome from tergite 2. Scale bars: **A, B** & **F** = 200 µm; **G** = 100 µm; **E** = 50 µm; **D** = 40 µm; **C, J** & **K** = 20 µm; **H** & **I** = 10µm.

projection shorter than claw, 8 ornamental trichomes *c* each side. Antennal articles VI and VII with distinctive notched appearance at distal edge, arrangement of sensilla in article VI with setiform sensillum anterior to 3 basiconic sensilla. Number of setae

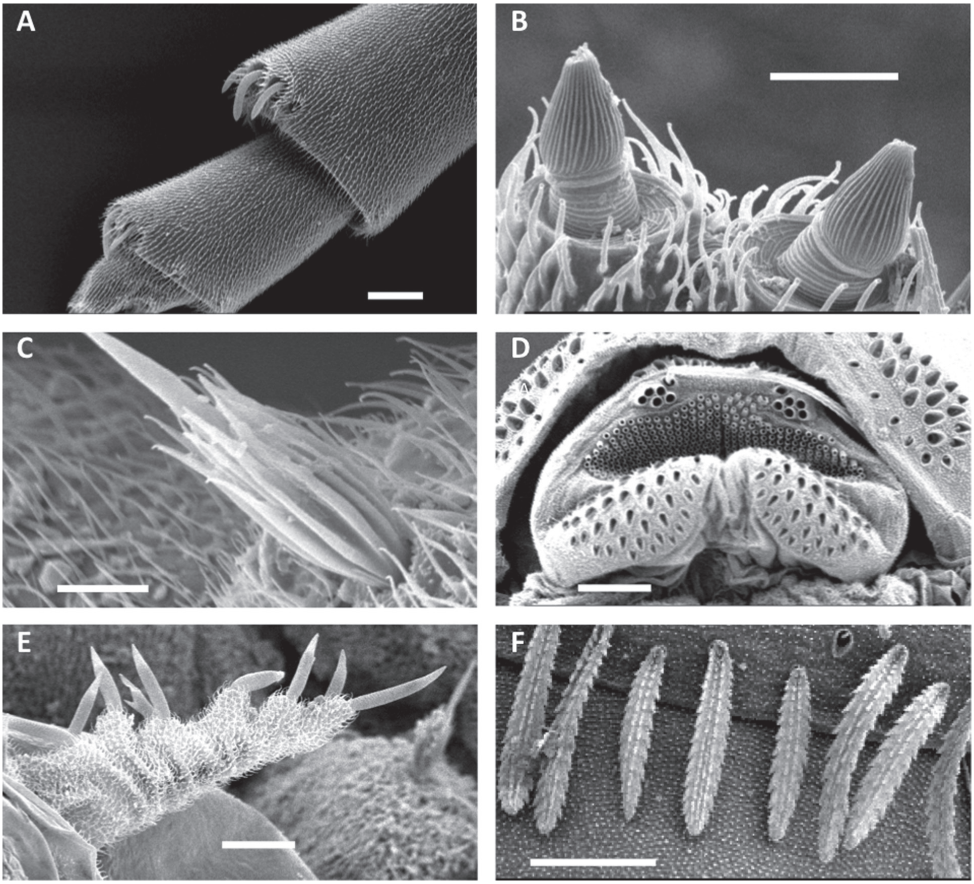

Figure 5. *Unixenus karajinensis* sp. n., Wittenoom **A** Antennal articles VI and VII showing notched distal edge **B** Detail of sensory cones on tip antennal article VIII **C** Ridged funicle of seta of coxa, prefemur and femur **D** Posterior view of stadium VII subadult, showing caudal trichome and associated trichome insertions **E** Lateral palp of gnathochilarium showing simple sensilla **F** Detail of tergal barbed trichomes. Scale bars: **A** = 20 µm, **B** & **C** = 5 µm, **D** & **F** = 50 µm, **E** = 20 µm.

on coxae 3–13 varies more widely from 1–6 in contrast to 2–3 in *U. mjoebergi*. The hooked caudal trichomes have double barbs proximal to the hooks, last sternal plate with 2 setae.

Etymology. The Australian aboriginal Banyjima people's name for the Hamersley Ranges is Karajini.

Description. Length of both sexes 2.8–3.3 mm, caudal bundle 0.3 mm.

Colouration in alcohol both sexes yellow brown with trichomes including caudal bundle medium to dark brown, long ornamental trichomes darker.

Head with 8 ocelli each side: 4 dorsal, 4 lateral (1 anterior, 2 medial and 1 posterior). Vertex with 2 posterior groups of trichomes arranged in 3 oblique rows (Fig. 4A). Number of trichomes varies, holotype with 40+38 trichomes, comprising: 8+8 (anterior rows), 21+20 (middle rows) and 11+10 (posterior rows). Total numbers each side varied: Tom Price paratypes 27–45 (n = 7); Wittenoom paratypes 25–35 (n = 6). Overall, both sexes showed highly variable number vertex trichomes (51–85), with asymmetrical pattern of 1–2 trichomes difference (occasionally up to 5). Trichobothria equal in size, arranged in shape of isosceles triangle with greater width *a–c*.

Antennae with proportions of 8 articles and 4 sensory cones typical of other species in genus (Figs 4B and 5B). Antennal articles VI and VII with distinctive notched appearance at distal edge (Fig. 5A), article VI with 3 thick basiconic sensilla of equal length, coeloconic sensillum posterior to basiconic sensilla, setiform sensillum anterior to basiconic sensilla. Antennal segment VII typical of genus with 1 coeloconic sensillum to the posterior followed anteriorly by 2 thick basiconic sensilla of similar height; 1 setiform sensillum between the basiconic sensilla (Fig. 4C). Clypeo-labrum with 12 setae along posterior margin, anterior margin with no dentition lateral to median cleft, two thirds surface covered with spherical papillae, papillae reducing in size to the posterior margin and lacking hairs (Fig 4D). Gnathochilarium with length lateral palp at least 2 X diameter medial palp; lateral palp with 13 cylindrical sensilla, medial palp 21 sensilla (Figs 4E and 5E).

Collum with almost symmetrical arrangement of trichomes, posterior row of trichomes with broad medial gap, extending to lateral clusters of trichomes, anteriorly a narrowing band of trichomes extending from each lateral cluster towards midline, scattered trichomes between posterior row and anterior bands (Fig. 4F). Number of trichomes 56L + 59R in holotype male; variation common with range per side: Wittenoom female paratypes 31–46 (n = 6); Tom Price paratype females 33–47 (n = 4), paratype males 37–43 (n = 3). Small lateral protuberances each with row of 6 forward-facing trichomes in holotype, varying between 4–6 in Tom Price and Wittenoom paratypes.

Trichomes of tergites 2–9 arranged on posterior half of tergite, 1 distinct posterior row with medial gap, merging with clusters laterally. Further trichomes anteriorly, loosely arranged in 2 to 3 rows. Anterior trichomes directed towards head while remaining trichomes directed posteriorly. Number of trichomes on tergite 2 in holotype male 61L+54R, variation common with range per side: Wittenoom paratype females 36–49 (n = 4); Tom Price paratype females 38–64 (n = 3), paratype males 49–52 (n = 2). Tergite 10 with wide medial gap, large clusters of trichomes either side, posterior rows forming part of each cluster (Fig 4F). Conical pleural projections along each side associated with tergites 2–10, each with dense cluster of trichomes. Tergal and pleural trichomes, long and thin with 2 internal rows projections (Figs 4L and 5F), tergal trichomes increasing in length posteriorly.

Legs 1 and 2 without trochanter, leg 1 also lacks tarsus 1. Trochanter and postfemur and tarsus 1 lack setae. Chaetotaxy as follows: coxa 1, 1 seta, coxa 2, 2 setae, coxae 3–13, 1-6 setae; prefemur, tibia and tarsus 2 with 1 seta, femur with 2 setae (Fig. 4G). Last sternal plate with 2 setae. Coxa, prefemur and femur with bi-articulate setae similar to those for *U. mjoebergi* with longitudinal ridges on basal funicle, each ridge extending distally in a long, thin projections which surround the base of the flagellum (Figs 4H and 5C), setae of tibia and tarsus 2 setiform. Telotarsus bearing anterior spinous projection shorter than claw which bears posterior and anterior processes, large lamella process present (Fig. 4I). Males with 6 pairs coxal glands, leg pairs 6–11.

Telson with ornamental trichomes arranged almost symmetrically with 5 trichomes (some variants with 4–8) *a*, 1*b*, and 8*c* each side of midline (Fig. 4J). Insertion points vary in size with *a* and *c* bigger than *b*. Single caudal bundle of hooked trichomes with 2–4 hooks and barbed stems. Double barbs of stem showing both distal- and proximal-facing barbs (Fig. 4K). Double barbs start immediately below hooks on 4 hook trichomes, with simple distal-facing barbs before first double barb on 2–3 hook trichomes. In immature stadia, two clusters of short barbate trichomes with same structure as ornamental trichomes *a* found ventral to caudal bundle (Fig. 5D). These clusters also observed in other species in the family Polyxenidae. They displace laterally after moulting to become pleural projections**.**

Distribution. This species is only known from three sites in the Hamersley Ranges, WA (Fig. 8). Both males and females were collected at each location. Since collection, the asbestos mine at Wittenoom has been closed and access restricted to the area that includes the type locality.

Remarks. The widespread distributions of *U. attemsi*, *U. mjoebergi* and *U. corticolus* sp. n. are not unexpected as their small size, bristles and very light weight make it probable that they are blown by the wind or become attached to bird feathers. Unexpectedly *U. karajinensis* sp. n. appears to be limited to a single mountain range, although further sampling may extend the distribution.

Unixenus corticolus sp. n.

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http://species-id.net/wiki/Unixenus_corticolus
Figs 1D, 6, 7

Holotype. Male, Deep Lead Flora and Fauna Park, near Stawell, Vic, 37°00'38"S, 142°44'19"E, 17 June 2005, M. Short and C. Huynh, in *Eucalyptus* bark, mounted on slide, MV K-11507.

Paratypes. One male, five females, same data as holotype, mounted on slides, male: MV K-11508, females 1–5: MV K-11509–13; one male and one female from Tidal River, Wilson's Promontory, Vic, 39°01'54"S, 146°18'49"E, 1 November 2005, C. Huynh, in *Melaleuca* bark, mounted on slides, male: MV K-11514, female: MV K-11515.

Other material examined. All collected by M. Short and C. Huynh: Ararat Hills Park, Vic, 37°14'30"S, 142°54'30"E, 17 June 2006, in *Eucalyptus* bark; Flinders, Vic, 38°28'54"S, 145°01'22"E, 25 August 2011, in *Melaleuca* bark; Pt. Addis, Vic, 38°22'27"S, 144°14'42"E, 21 August 2011, in *Eucalyptus* bark; Tathra, NSW, 36°43'35"S, 149°59'8"E, 30 December 2007, in *Melaleuca* bark; Lakes Entrance, Vic, 37°52'45"S, 146°10'49"E, 28 December 2007, in *Melaleuca* bark; Sunnyside Beach, Mornington, Vic, 38°12'06"S, 145°03'41"E, 17 May 2008, in *Melaleuca* bark;

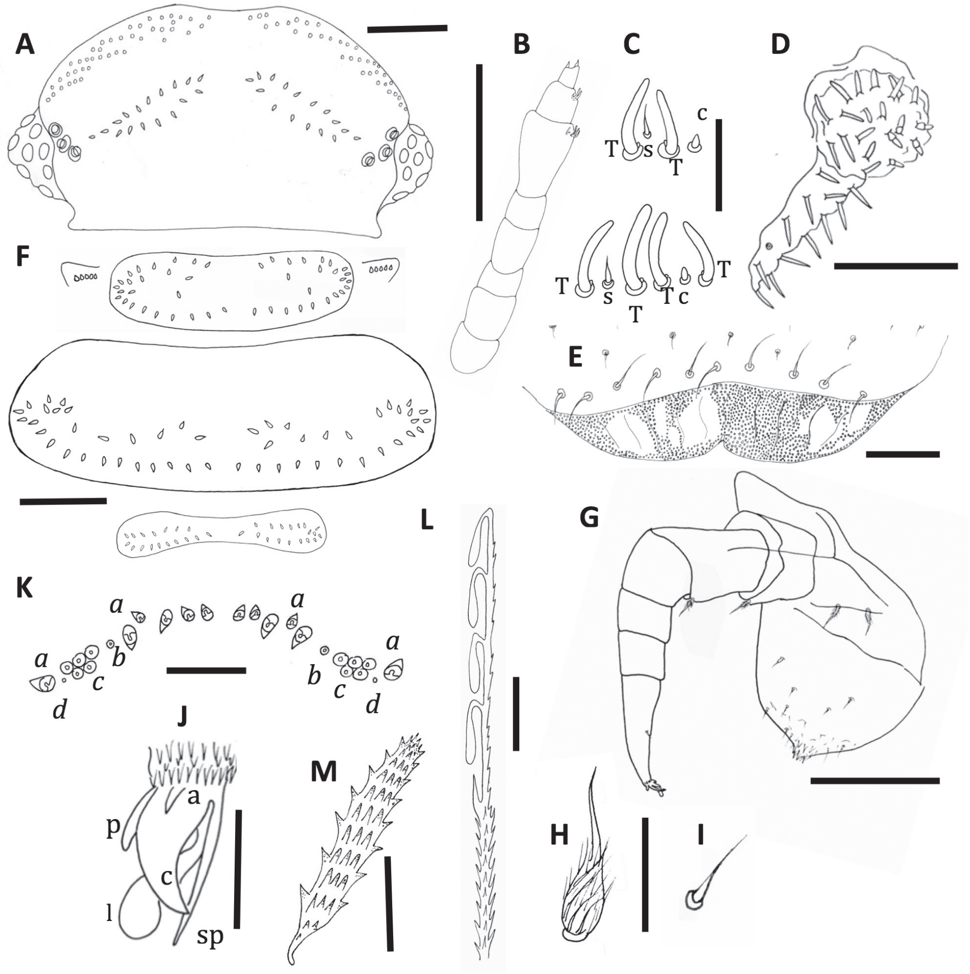

Figure 6. *Unixenus corticolus* sp. n., holotype, MV K-11507 **A** Head, dorsal view showing arrangement of ocelli, position of trichobothria and trichome insertions **B** Right antenna **C** Details of sensilla on antennal articles VI and VII, sensilla type indicated as follows: coeloconic (c), setiform (s), thick basiconic (T), article VI sensilla lower row **D** Left palp of gnathochilarium showing long lateral palp, medial palp and simple sensilla **E** Clypeo-labrum **F** Collum, tergite 2 and tergite 10 showing pattern of trichome insertions **G** Leg 2 showing chaetotaxy and penis **H** Details of seta of coxa, prefemur and femur **I** Setiform sensillum, tarsus 2 **J** Anterior view of telotarsus showing anterior spinous projection (s), claw (c) with anterior (a) and posterior (p) processes and lamella (l) **K** Pattern of ornamental trichomes *a, b, c* and circular indentations *d* **L** Hooked caudal trichome **M** Tergal trichome. Scale bars: **B** & **G** = 150 µm; **A** & **F** = 100 µm, **D** = 50 µm, **E** & **K** = 40 µm; **M** = 30 µm; **C**, **H** & **I** (shared bar), **J** & **L** = 10 µm.

Launceston, Tas, 41°27'S, 147°09'E, 1 February 2008, in private garden, bark mulch; Holey Plains State Park, Vic, 38°13'S, 146°53'E, 20 April 2008, exuviae, in *Eucalyptus* bark; Narawntapu National Park, Tas, 42°55'07"S, 147°49'23"E, 31 January 2008, in *Eucalyptus* bark; Cataract Gorge, Launceston, Tas, 41°27'S, 147°09'E, 1 February 2008, in *Eucalyptus* bark.

Etymology. Adjective; this species is almost always found under bark, rarely in litter, in contrast to other species in the genus.

Diagnosis. 4 basiconic sensilla on antennal article VI, basiconic sensillum 4 posterior to coeloconic sensillum, short lateral palps on gnathochilarium (1.5 X diameter of medial palp), 1 seta on femur and no setae on tibia, 5 ornamental trichomes *c* each side, caudal hooked trichomes with 3–6 hooks. Setae of coxa, prefemur and femur similar to that of *U. mjoebergi* but with slightly curving and fewer ridges on funicle.

Description. Length of both sexes 2.4–3.4 mm, caudal bundle 0.45 mm.

Colour dark grey with broad unpigmented band dorsally along midline. Trichomes unpigmented, appearing translucent white in live specimen, long ornamental trichomes *c* darkly pigmented in contrast to trichomes of caudal bundle that appear white in live specimens.

Head (Fig. 6A) with 8 ocelli each side: 4 dorsal, 4 lateral (1 anterior, 2 medial and 1 posterior). Vertex with 2 posterior groups of trichomes arranged in 2 oblique rows, separated medially by a broad space. Number of trichomes varies, holotype with 10L+10R (anterior rows) and 7L+6R (posterior rows), total each side 17L and 16R. Number of trichomes per side varies 14–18, with no differences between sexes, asymmetrical pattern common with maximum difference 2 trichomes. Trichobothria equal in size, arranged in triangle with angle at b >120°, distance a–b slightly shorter than distance b–c.

Antennae with proportions of 8 articles and 4 sensory cones typical of other species in genus *Unixenus* (Fig. 6B). Antennal article VI with 4 thick basiconic sensilla of equal length, coeloconic sensillum between basiconic sensilla 3 and 4, setiform sensillum between basiconic sensilla 1 and 2. Antennal segment VII typical of genus with 1 coeloconic sensillum to the posterior followed anteriorly by 2 thick basiconic sensilla of similar height; 1 setiform sensillum between the basiconic sensilla (Figs 6C and 7B). Clypeo-labrum covered in small spherical papillae typical of genus *Unixenus,* anterior edge with median cleft, no lamella teeth, posterior margin 8–10 setae (Fig. 6E). Gnathochilarium with length lateral palp 1.5 X diameter of medial palp. Lateral palp with 13 cylindrical sensilla, medial palp 21 sensilla (Fig. 6D).

Collum with almost symmetrical arrangement of trichomes, 2 main rows of trichomes each side of medial gap with small number of trichomes between rows, rows linked laterally with small cluster of trichomes (Fig. 6F). Trichomes 24L+23R in holotype, varying between 23–28 per side in paratypes. Small lateral protuberances each with row of 5 forward facing trichomes in holotype, 3–7 in paratypes. In tergites 2–10, trichomes arranged on posterior half of tergite in 2 loose rows with small clusters laterally (Fig. 6F). Trichomes in anterior row directed towards head, those of posterior row directed posteriorly. Trichome number on tergite 2 variable: 26L+26R in holotype, 24–34 each side in paratypes. Conical pleural projections along each side associated with tergites 2–10. Tergal trichomes, all short, barbate and thicker than those of *U. mjoebergi* and *U. karajinensis* with three internal longitudinal rows of projections (Figs 6M and 7C), pleural trichomes slightly longer.

Legs 1 and 2 without trochanter, leg 1 also lacks tarsus 1. Trochanter, postfemur, tibia and tarsus 1 lack setae. Chaetotaxy as follows: coxa 1, one seta, coxa 2–3, 2–3

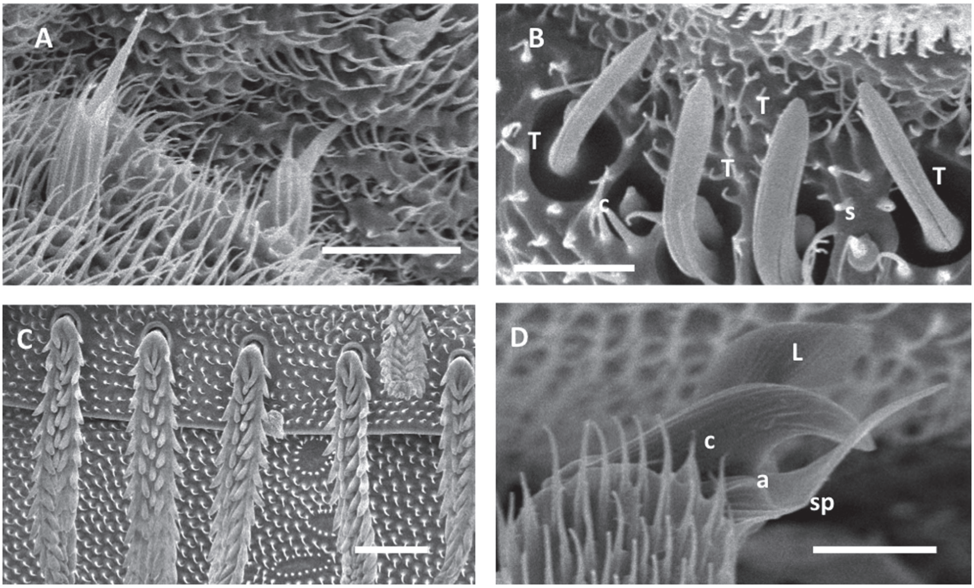

Figure 7. *Unixenus corticolus* sp. n., adult male, Deep Lead **A** Setae on coxa **B** Sensilla on antennal article VI, thick basiconic (T), coeloconic (c), setiform (s) **C** Detail of barbed tergal trichomes **D** Detail of telotarsus showing anterior spinous projection (sp), claw (c), lamella process (l), anterior process (a). Scale bars: **A** = 10 µm, **B** & **D** = 5 µm, **C** = 20 µm.

setae, coxa 3–10, 2–4 setae, coxa 13, 0–2 setae (males 2 setae, females 0 setae); prefemur, femur, and tarsus 2 with single seta (Fig. 6G). Structure of setae of coxa, prefemur, femur similar to that of *U. mjoebergi* and *U. karajinensis* but with less ridging on funicle and fewer projections, ridging slightly curved around funicle (Figs 6H and 7A); seta of tarsus 2 setiform (Fig. 6I). Telotarsus bearing anterior very thin spinous projection longer than claw. Claw bears anterior and posterior slender processes, large lamella process (Figs 6J and 7D). Males with 2 pairs coxal glands on leg pairs 8–9.

Ornamental trichomes of telson with 4–6 trichomes *a*, 1*b*, and 5*c* (comprising 2 long, 1 medium and 2 shorter dark brown barbate trichomes) each side of midline (Fig. 6K). Caudal bundle trichomes with 3–6 hooks, distal-facing barbs along the stem (Fig. 6L).

Distribution. Specimens of this species have been found at a number of locations some distance from each other in southern Australia (Fig. 8). Specimens were collected from under bark of *Eucalyptus*, *Melaleuca* and *Leptospermum.* The species was found once only on the ground, in litter formed from mulched bark. Males and females were collected at each location. No specimens were found in museum collections.

Remarks. Although this species is similar in many ways to *U. mjoebergi*, it appears to have different habitat requirements as it is rarely found in litter, and then only in bark mulch. This would explain the absence of the species from museum collections that are mainly the result of pitfall trapping or extraction from litter.

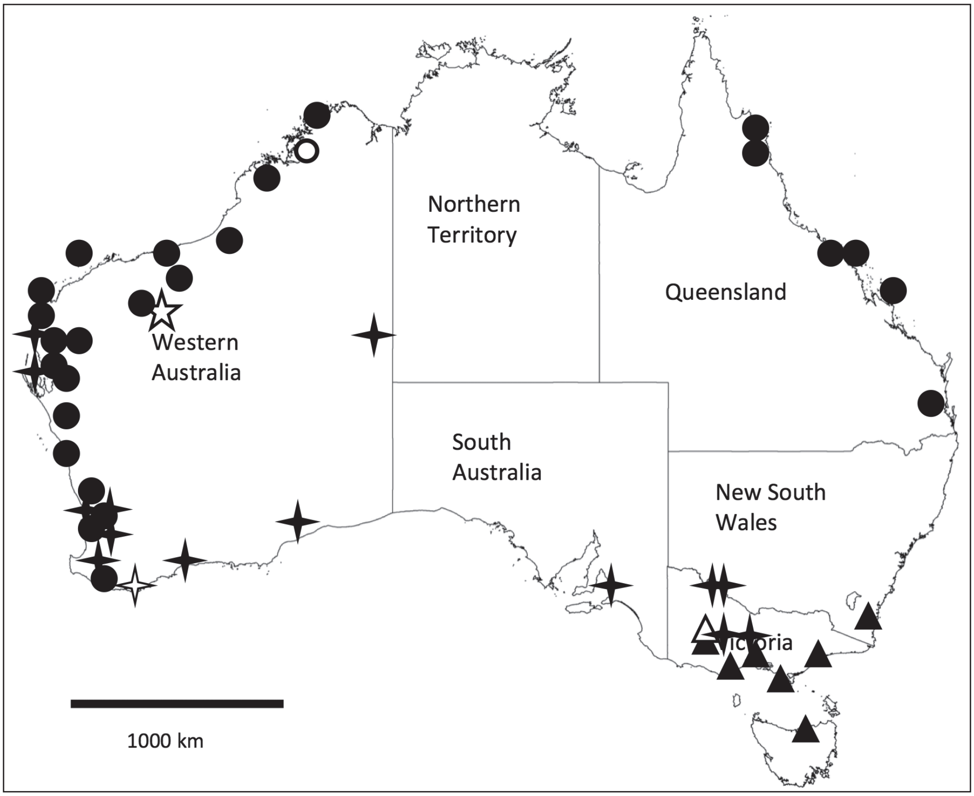

Figure 8. Map of Australia with states indicated, showing distribution of four *Unixenus* species. *U mjoebergi* (Verhoeff, 1924): filled circles, type region shown as open circle; *U. attemsi* Nguyen Duy-Jacquemin and Conde, 1967: filled 4 point star, type locality shown as open 4 point star; *U. karajinensis* sp. n.: type locality shown as open 5 point star; *U corticolus* sp. n.: filled triangles, type locality shown as open triangle.

Key to described species of *Unixenus*

The type species from India, *U. padmanabhii,* is not included as insufficient details are known:

1a. Presence of unridged setae on legs.. **2**
1b. Presence of leg setae with ridges and spiny projections................................ **3**
2a. One small seta on tibia of at least legs 1–5 .... ***U. broelemanni***
2b. No setae on tibia.. **4**
3a. Presence of 3 ornamental trichomes *c* per side.......................... ***U. mjoebergi***
3b. Presence of more than 3 ornamental trichomes *c* per side........................... **5**
4a. Telotarsus with more than 2 processes on claw............................ ***U. attemsi***
4b Telotarsus with 1 process only on claw.................................. ***U. vuillaumei***

5a. 5 ornamental trichomes *c* per side, 2 pairs coxal glands in male.................... .... ***U. corticolus*** **sp. n.**

5b. 8 ornamental trichomes *c* per side, 6 pairs coxal glands in male .... ***U. karajinensis*** **sp. n.**

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RESEARCH ARTICLE

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Barcoding Fauna Bavarica: Myriapoda – a contribution to DNA sequence-based identifications of centipedes and millipedes (Chilopoda, Diplopoda)

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Abstract

We give a first account of our ongoing barcoding activities on Bavarian myriapods in the framework of the Barcoding Fauna Bavarica project and IBOL, the International Barcode of Life. Having analyzed 126 taxa (including 122 species) belonging to all major German chilopod and diplopod lineages, often using four or more specimens each, at the moment our species stock includes 82% of the diplopods and 65% of the chilopods found in Bavaria, southern Germany. The partial COI sequences allow correct identification of more than 95% of the current set of Bavarian species. Moreover, most of the myriapod orders and families appear as distinct clades in neighbour-joining trees, although the phylogenetic relationships between them are not always depicted correctly. We give examples of (1) high interspecific sequence variability among closely related species; (2) low interspecific variability in some chordeumatidan genera, indicating that recent speciations cannot be resolved with certainty using COI DNA barcodes; (3) high intraspecific variation in some genera, suggesting the existence of cryptic lineages; and (4) the possible polyphyly of some taxa, i.e. the chordeumatidan genus *Ochogona*. This shows that, in addition to species identification, our data may be useful in various ways in the context of species delimitations, taxonomic revisions and analyses of ongoing speciation processes.

Keywords

Chilopoda, Diplopoda, COI barcoding, Bavaria, Germany

Introduction

Molecular species identification based on sequence diversities in the Folmer segment of mitochondrial COI DNA has been under intense study for some years (Hebert et al. 2003, Savolainen et al. 2005). For the identification of a wide set of species, reference barcode libraries are needed; therefore, various projects are currently building such libraries by mass sequencing. The Barcoding Fauna Bavarica project (http://www.faunabavarica.de, Haszprunar 2009, Hausmann et al. 2011a, b), in close association with IBOL, the International Barcode of Life (http://ibol.org/), the DNA bank facility at Zoologische Staatssammlung München (ZSM) (http://www.zsm.mwn.de/dnabank/, Gemeinholzer et al. 2011), and the GLOMYRIS project of the Global Biodiversity Information Facility (GBIF) (http://www.gbif.de/evertebrata2/glomyris), aims to barcode all animal species in Bavaria, i.e. some 35000 species, representing 85% of the species found in Germany.

Among the Chilopoda and Diplopoda, the 146 species known from Bavaria cover 73% of the fauna of Germany. Hence, the first aim of our study is to establish a barcode reference library for Bavarian Myriapoda that will be expanded step by step (the dataset treated in this paper can be accessed in Barcode of Life Data Systems (BOLD; Ratnasingham and Hebert 2007, http://www.boldsystems.org) under 'MYFBA (Fauna Bavarica Myriapoda public 1)' as part of the campaign 'Fauna Bavarica'). Moreover, myriapods found in our studied area cover many of the numerous species and subspecies of uncertain morphology-based species delimitations described in the huge works of K.W. Verhoeff (e.g. 1934, 1935) and C. Attems (e.g. 1927) many of which need taxonomic revision. Our work aims to provide morphology-independent sets of characters to enable us to check against the descriptions and species delimitations, and therefore to draw new conclusions about the validity of these species. This is also important since in the Bavarian Alps numerous species are found which are relicts of speciation processes that occurred during and after the last glaciation periods. Our barcodes will provide a basis or a test for these analyses (e.g. Pilz et al. 2008). Furthermore, barcoding of myriapods is of particular interest since in many species, i.e. in many diplopods such as the family Julidae, only a small fraction of the specimens (only adult males) can be identified using morphological sets of characters. Conversely, DNA barcoding allows the determination of all developmental stages from the egg to the male or female adults. In the future, DNA barcoding will therefore allow the identification of all life stages of these taxa instead of adult males only.

In the present paper we give an overview of our ongoing barcoding activities, which so far cover 73% of all Bavarian Chilopoda and Diplopoda. In addition to conventional analysis of the actual dataset based on our BOLD data, we give examples of how our barcodes will contribute to taxonomic revisions and to analyses of past and ongoing speciation processes.

Material and methods

Sampling

To cover the variability within species, numerous samples from locations inside and outside Bavaria have been included. Besides the centipedes and millipedes known to occur in Bavaria, species that might be found there in the future have also been included, as well as close relatives of the known Bavarian species (Fig. 1). Since the study arose from the 'Barcoding Fauna Bavarica' project, sampling was restricted to a few individuals per species. We have tried to include material from all four major Bavarian faunal regions as defined in Voith (2004), provided that the species occurred in all of them. If this was not the case, additional sampling took place in adjacent countries. Attempts to amplify and sequence museum material (stored in denatured 75% ethanol), mainly from the more than 70 year-old Verhoeff collection housed at the ZSM, have failed. Hence we had to use newly sampled material less than two years old. This fresh tissue material was ideally stored in reagent-grade 96% ethanol which was exchanged several (3-4) times. In practice it was sometimes unavoidable to use material stored in about 75% ethanol for some days or months before replacement with 96% ethanol. All specimens used for sequencing have been photographed, as required by the Canadian Centre for DNA Barcoding (CCDB). Most of these photos were taken of live specimens in the field and are available online via BOLD. Taxonomy and nomenclature is based on the Bavarian list by Spelda (2006), except for a few updates reflecting more recent taxonomic decisions, e.g. taxa raised to species rank, new synonymies and new combinations. A website has been established for 'Barcoding Fauna Bavarica' (http://www.fauna-bavarica.de) that continuously updates project progress, such as lists of species and their barcode coverage.

DNA sequencing

Sequencing was carried out at the CCDB, using the standard protocols of IBOL (http://www.dnabarcoding.ca/pa/ge/research/protocols). For reasons of performance, so far only the C_LepFolF and C_LepFolR primers have been used for PCR and sequencing. Barcoded voucher specimens are stored at ZSM, and DNA extracts from the specimens at the CCDB and the ZSM's DNA bank facility (http://www.zsm.mwn.de/dnabank/). Specimen data, images and DNA sequences will be available on BOLD. BOLD numbers are given for each specimen in the depicted NJ trees (Figs. 8–11). These allow the tracking of our sequences in BOLD and GenBank, respectively.

Sequencing failed for about 30% of the species. Sometimes whole genera (*Trachysphaera*, *Ommatoiulus*, *Megaphyllum*, *Mycogona*), and sometimes species-level taxa

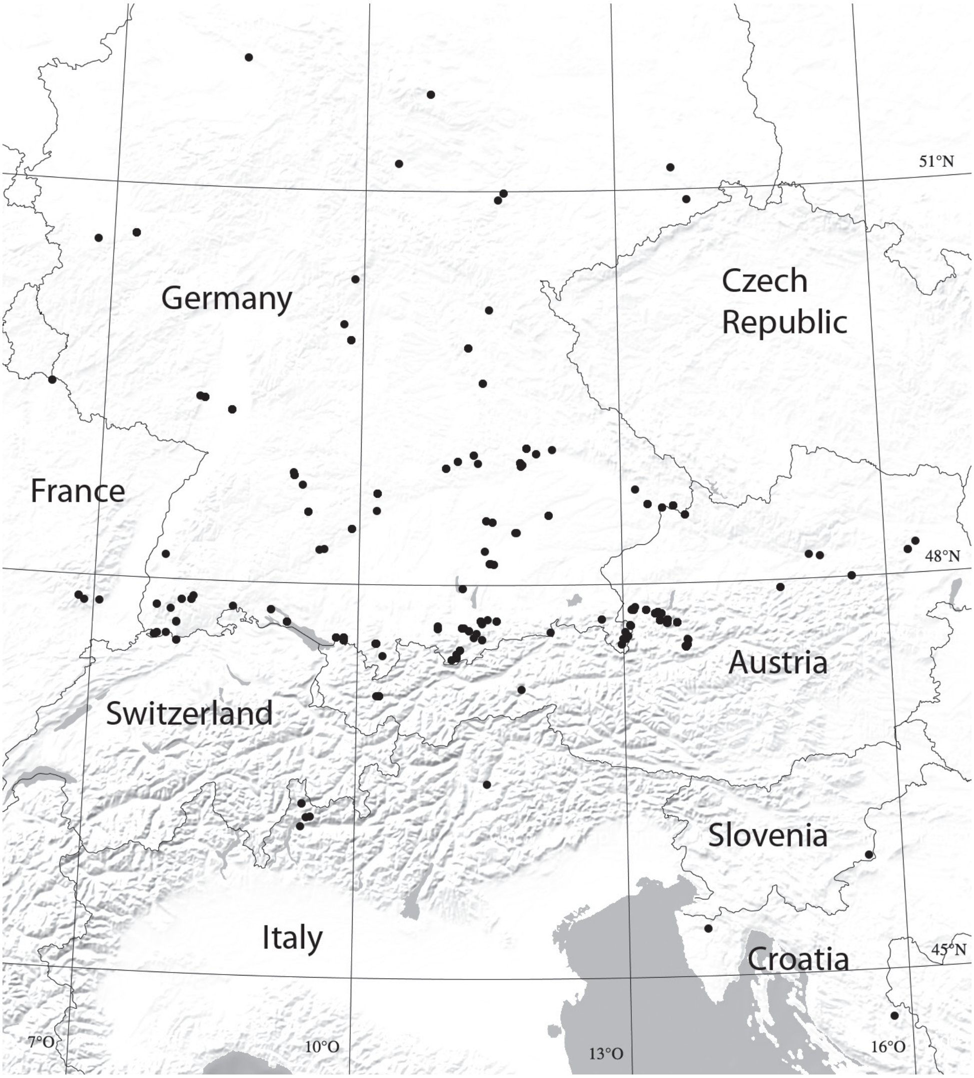

Figure 1. Map of sampled areas (dots). For checks of intraspecific variability of COI sequences, localities in Bavaria, but also elsewhere within the species' areas of distribution, have been sampled and analyzed (sampling data from November 2008 to November 2010; a few specimens from northern Spain omitted).

(*Glomeris undulata* s.l., *Leptoiulus simplex*-group) were reluctant to barcoding. In these cases we either obtained no barcodes, or less than a quarter of the specimens were successfully barcoded. Hence, barcoding success of single samples was somewhat unpredictable. It seems that minor differences in tissue composition and protocol determine whether or not a sample runs; e.g., in one particular plate all *Megaphyllum* and *Ommatoiulus* were amplified successfully, whereas they had failed before.

Data analysis

Resulting data for the myriapods treated here are taken from the respective tools included in BOLD, and calculated using the Kimura 2 Parameter (K2P) model. Sequences were imported into PAUP* (Swofford 2003) as Fasta files, and tree statistics were calculated using the bootstrap algorithm of PAUP* with 10 replicates and a neighbour-joining/UPGMA search. Only groups with a frequency above 50% were retained for consensus tree reconstruction.

Results

Data analysis

At the moment our myriapod barcode library includes 320 specimens, 122 species, 56 genera and 24 families of Myriapoda (Fig. 2). All sequences were longer than 500 bp and thus fulfill the requirements for barcoding. The following analysis is based on this dataset (MYFBA), composed of a total of 126 taxa (122 species, three additional subspecies and one subspecies hybrid).

The mean sequence compositions in our sequences are G = 16.32%, C = 21.75%, A = 30.04% and T = 31.87% in Chilopoda, and G = 17.64%, C = 17.67%, A = 26.21% and T = 38.29% in Diplopoda. This shows a pronounced bias towards A and T, which is characteristic of arthropods.

In Chilopoda (Fig. 3) the lowest interspecific distance (K2P distance to nearest neighbour) was found between the species *Lithobius borealis* and *Lithobius valesiacus* (11.99%), and the maximum between *Pachymerium ferrugineum* and *Strigamia crassipes* (25.26%). The mean value of the interspecific distance for Chilopoda was 18.30%. Interspecific distances in Diplopoda (Fig. 4) ranged between 0 % in the subspecies of *Craspedosoma rawlinsii* (including the taxa *alemannicum, alsaticum, transsilvanicum* and the hybrid *germanicum* (= *alemannicum X rawlinsii*)) and 33.18 % between the neighbour pair *Polyzonium germanicum* and *Geoglomeris subterranea* which belong to different orders. The mean value of the interspecific distance for Diplopoda was 14.17%.

Intraspecific distances in Chilopoda (K2P maximum pairwise distance) ranged between 0%, for five species, and 21.55% for *Lithobius microps*, with a mean value for all studied chilopod species of 6.73% (Fig. 5). In Diplopoda, 0% was found for 19 species, and the maximum was 6.61 in *Glomeridella bitaeniata*, with a mean value of 0.82% for all studied diplopods (Fig. 6).

Neighbour-joining trees

Analysis of our data resulted in the Neighbour-joining (NJ) trees shown in Figs 7–11. Especially in Diplopoda-Helminthomorpha, where species delimitation is compara-

Figure 2. Some Bavarian myriapods for which barcodes are now available. A. *Glomeris pustulata* Latreille, 1804. B. *Polydesmus helveticus* Verhoeff, 1894. C. *Cylindroiulus boleti* (C. L. Koch, 1847). D. *Unciger foetidus* (C. L. Koch, 1838). E. *Haasea flavescens* (Latzel, 1884). F. *Atractosoma meridionale* Fanzago, 1876. G. *Cryptops parisi* Brölemann, 1920. H. *Henia vesuviana* (Newport, 1845). Photos: J. Spelda.

tively easy due to the diversity of their species-specific secondary copulatory apparatus (gonopods), the results of classical (morphological) taxonomy correspond perfectly with the COI lineages in most cases of our dataset. In the following, we give examples to show how fruitful the combination of barcoding and classical taxonomy can be in myriapod research.

Though the mitochondrial COI gene is generally not seen as adequate for resolving relationships at taxonomic levels higher than species or genus, all barcoded myriapod

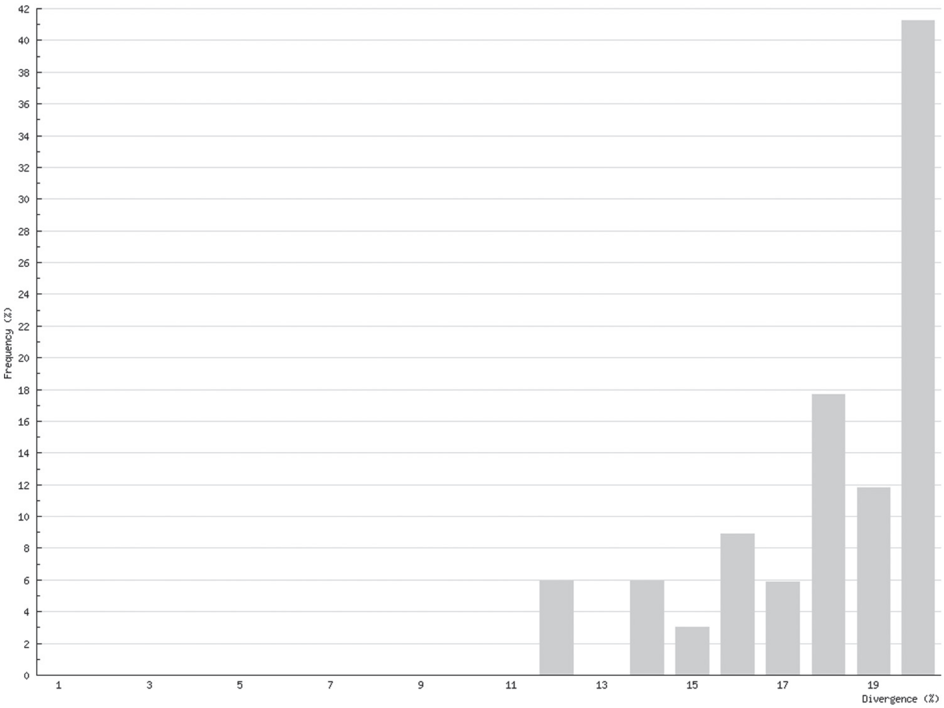

Figure 3. Interspecific COI variability (K2P): distance to nearest neighbour; Chilopoda

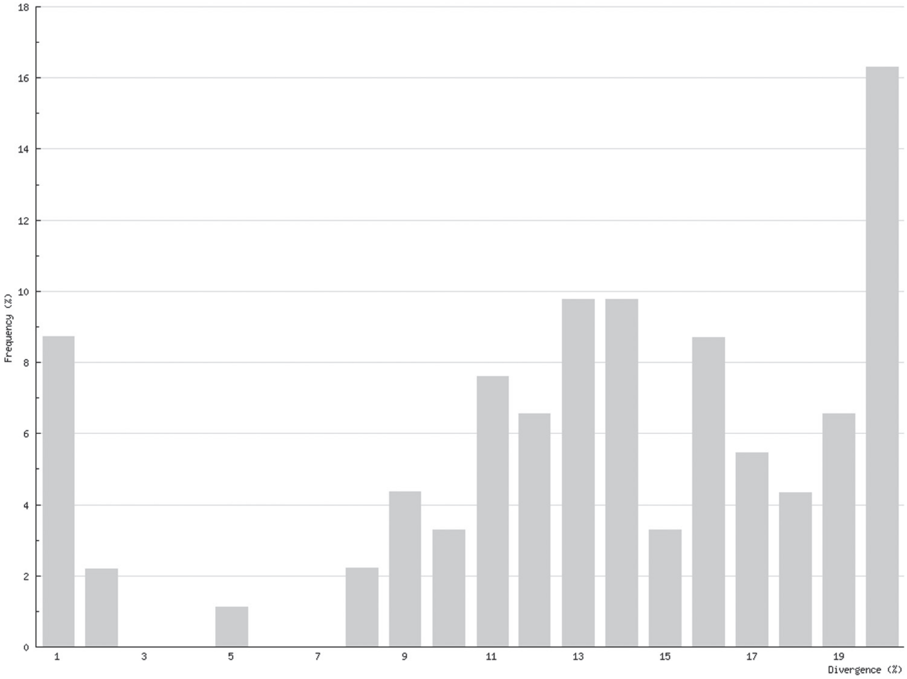

Figure 4. Interspecific COI variability (K2P): distance to nearest neighbour; Diplopoda

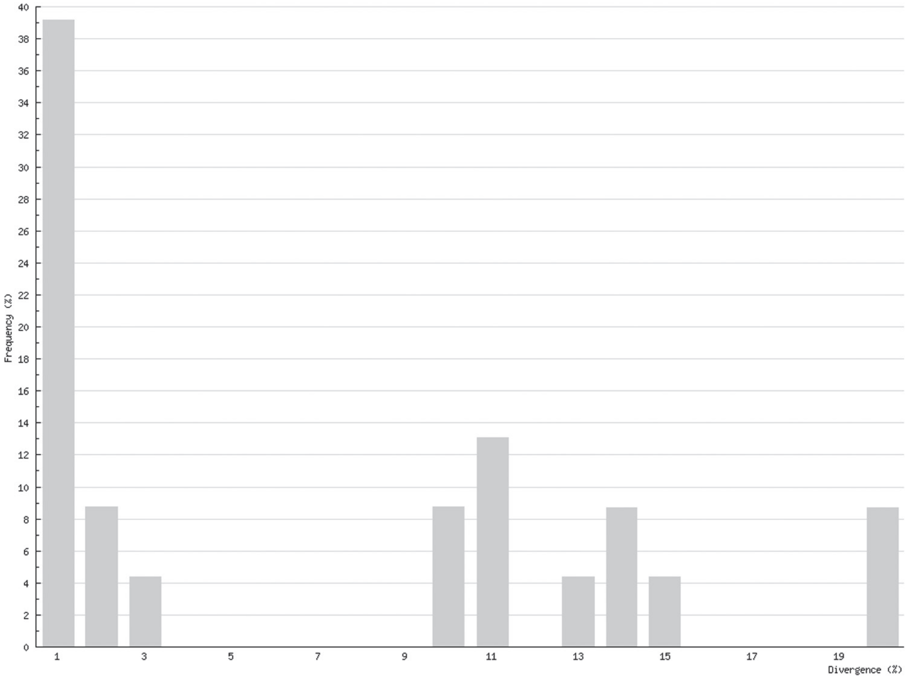

Figure 5. Intraspecific COI variability (K2P): maximum pairwise distances; Chilopoda

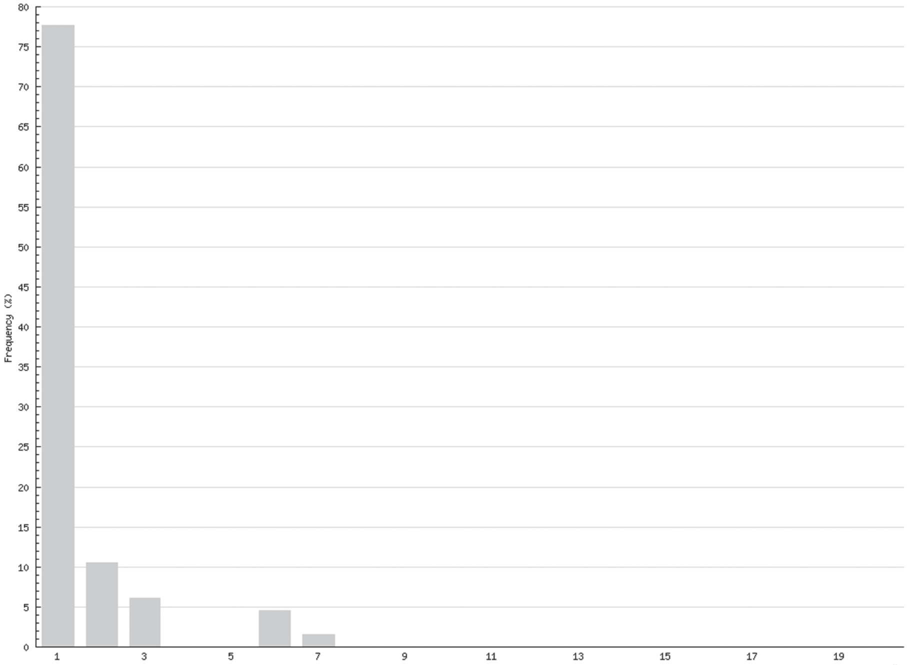

Figure 6. Intraspecific COI variability (K2P): maximum pairwise distances; Diplopoda

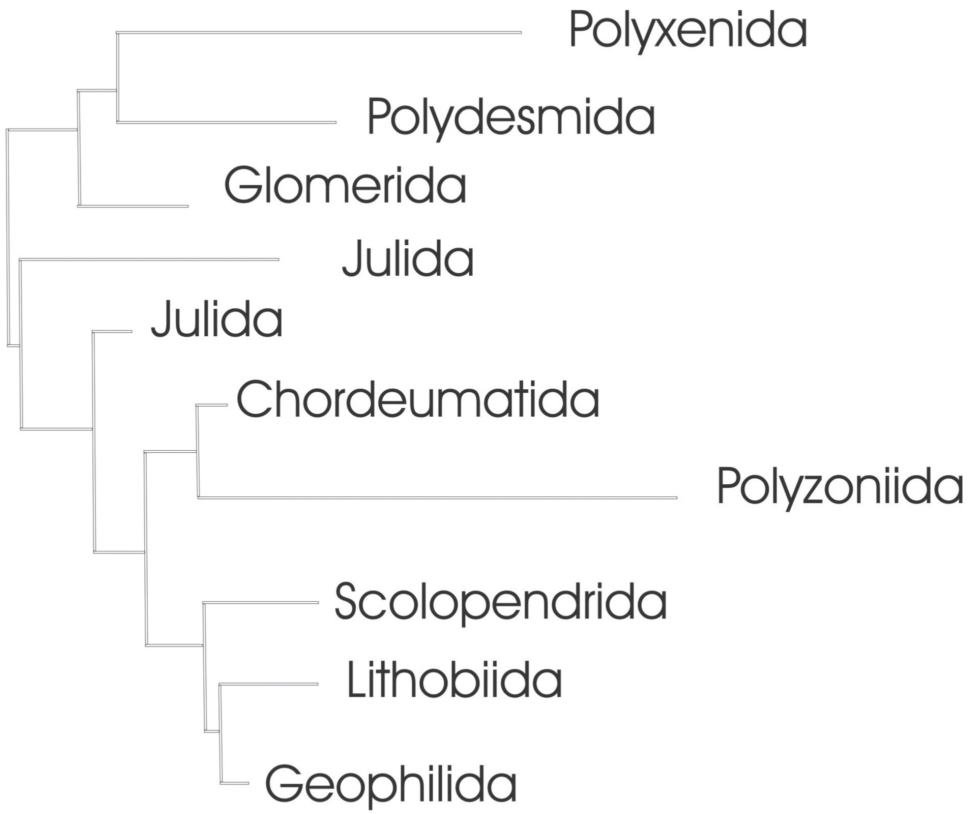

Figure 7. Complete neighbour-joining tree of COI sequence divergences (K2P model) of studied myriapod orders; barcoded terminal taxa and clades above their basal nodes omitted. This tree serves for orientation in the detailed trees given in Figs 8–11.

orders (Polyxenida, Polydesmida, Glomerida, Chordeumatida, Polyzoniida, Scolopendrida, Lithobiida and Geophilida) form single COI clades, except for the Julida, which form two clades (Figs 7, 9). The latter is not too puzzling, however, as according to Enghoff (1981) one of these two clades is formed by the species *Nemasoma varicorne*, which belongs to the only distantly related superfamily Nemasomatoidea. Moreover, several chordeumatidan families are also well supported by the barcodes, i.e. Mastigophorophyllidae, Haaseidae and Craspedosomatidae (Fig. 10).

Most of the studied species appear as distinct COI clades. Barcoded species can overlap for two reasons. First, speciation may have been very recent, e.g. during Pleistocene glacial episodes, as is the case for the diplopod genera *Craspedosoma*, *Rhymogona* and *Listrocheiritium* (Spelda 1996). In these genera genetic introgression is thought to occur commonly. For that reason the subtaxa are treated as subspecies (see Hauser 2004 for *Craspedosoma*, and Scholl and Pedroli-Christen 1996 for *Rhymogona*) or as (semi)species when their separation has been confirmed (Spelda 2006 for *Rhymogona*). The second reason for overlapping barcode groups originates from

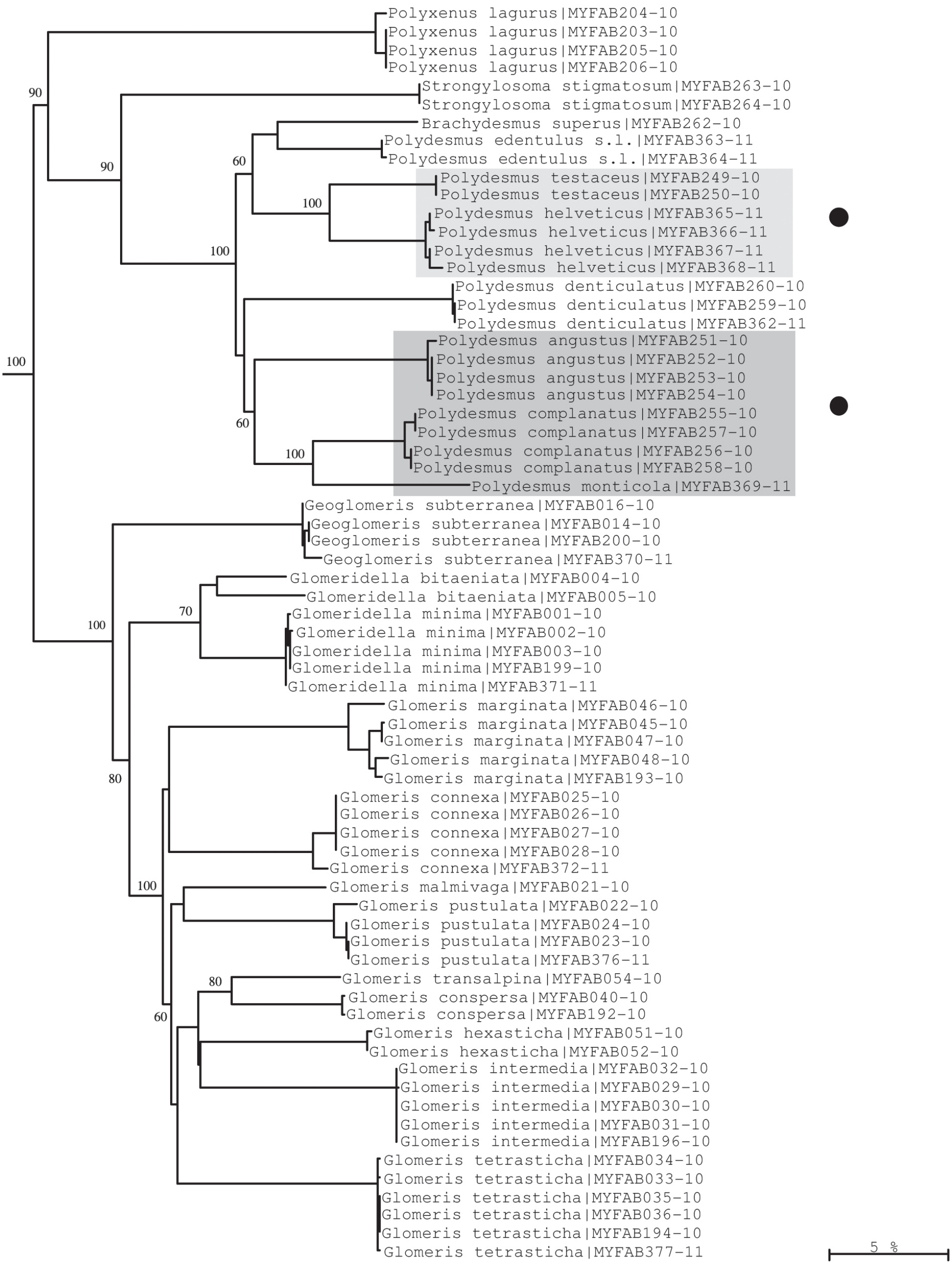

Figure 8. Neighbour-joining tree of COI sequence divergences (K2P model) of studied Polyxenida, Polydesmida and Glomerida. Solid circles: examples of excellent resolution of very close species of the genus *Polydesmus*. Numbers above and below branches show bootstrap values of NJ analysis, branch length indicates sequence divergence in %.

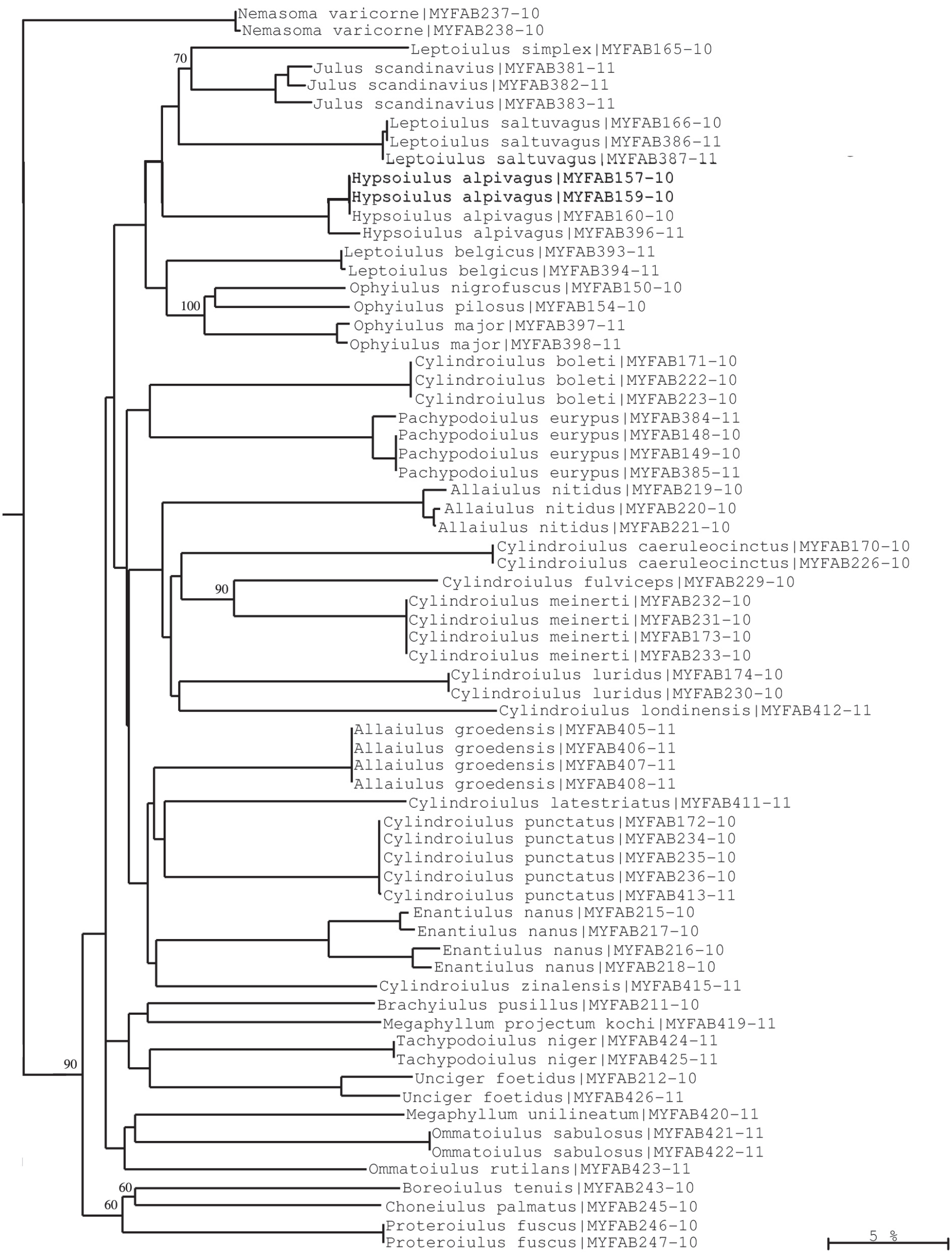

Figure 9. Neighbour-joining tree of COI sequence divergences (K2P model) of studied Julida. Note well-supported COI groups for each species allowing for sequence-based species identification. Numbers above and below branches show bootstrap values of NJ analysis, branch length indicates sequence divergence in %.

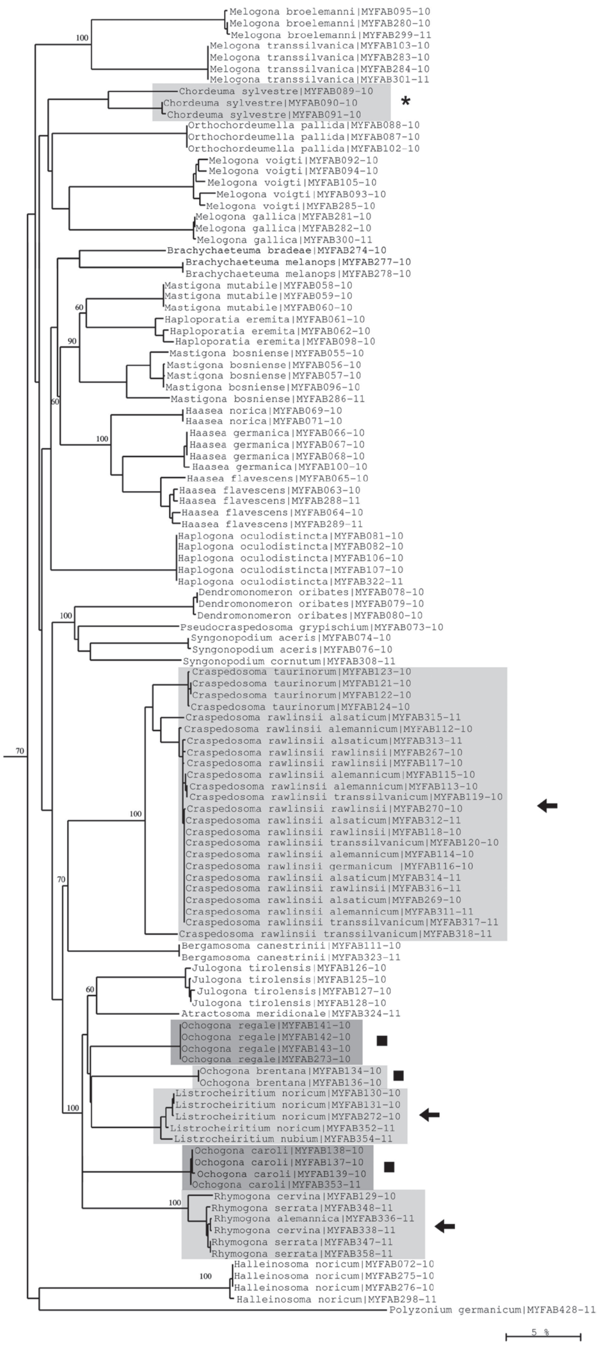

Figure 10. Neighbour-joining tree of COI sequence divergences (K2P model) of studied Chordeumatida. Asterisk: deep barcoding divergence in *Chordeuma silvestre*; solid squares: polyphyly of genus *Ochogona*; arrows: low sequence divergences in the genera *Craspedosoma*, *Listrocheiritium* and *Rhymogona*. Numbers above and below branches show bootstrap values of NJ analysis, branch length indicates sequence divergence in %.

extraordinarily high intraspecific variation (over 5% divergence) in several nominal chilopod species. Unfortunately, the chilopod samples in our dataset include a comparatively high number of singletons and doubletons, which makes it difficult to decide whether we face cryptic species or genuinely high intraspecific variation. For example, the genus *Strigamia*, especially the *S. crassipes* group, was previously split into many more species than today (Verhoeff 1935), a solution that might be justified in the light of our barcoding results.

At the species and genus levels, we found examples of both well and weakly supported species. For example, *Polydesmus testaceus* and *P. helveticus* (both often regarded as belonging to a separate genus *Propolydesmus*; see Enghoff and Golovatch 2003), and *P. angustus, P. illyricus* and *P. monticola* (*Polydesmus* s. str.), respectively, both form closely related species groups of highly similar morphology that show interspecific COI differences of more than 5%, and hence can be identified unequivocally using DNA barcodes (Fig. 8). It is also interesting to see that two other 'true' *Polydesmus* species, *P. denticulatus* and *P. edentulus*, are quite distant from both species groups, which implies that the genus *Polydesmus* could be split further.

Conversely, very low interspecific variation is found, e.g., in the chordeumatidan genera *Craspedosoma*, *Listrocheiritium* and *Rhymogona* (Fig. 10). In particular it was not possible to resolve the very closely allied species/subspecies complex within the *Craspedosoma rawlinsii* –group, a result that may reflect ongoing introgression and hybridization. The members of this group even exhibit nearest neighbour distance values of zero, indicating that the COI barcoding method is not suitable for separating its subtaxa.

Moreover, examples of high intraspecific variation can be found in several *Lithobius* species *(L. forficatus, L. mutabilis, L. tricuspis)* (Fig. 11), and in *Chordeuma sylvestre* (Fig. 10). These deep barcoding divergences could represent more than just high variation and might indicate that cryptic species, previously undetected using the classical morphological approach, are present among our samples. However, the revalidation of the species *L. glacialis* by Pilz et al. (2008) is clearly supported by our barcoding results. This species is distinctly separate from the lowland clade of *L. mutabilis* (a clade that might contain a cryptic species, as stated above), but shows only low intraspecific variation, even though the investigated material originates from very distant mountain areas (Wetterstein Mts Bavaria, and Dachstein Mts Austria). Surprisingly deep divergences are also found within the chordeumatidan genus *Ochogona*, suggesting that this genus is paraphyletic (Fig. 10).

Discussion

Despite the success of COI barcoding in so many species of centipedes and millipedes it has to be admitted that there are still technical problems with this method that make the success of the barcoding process for any single sample unpredictable. For reasons of cost efficiency the CCDB presently uses only one set of standard primers that are probably not optimal for all groups of centipedes and millipedes. For example, we have

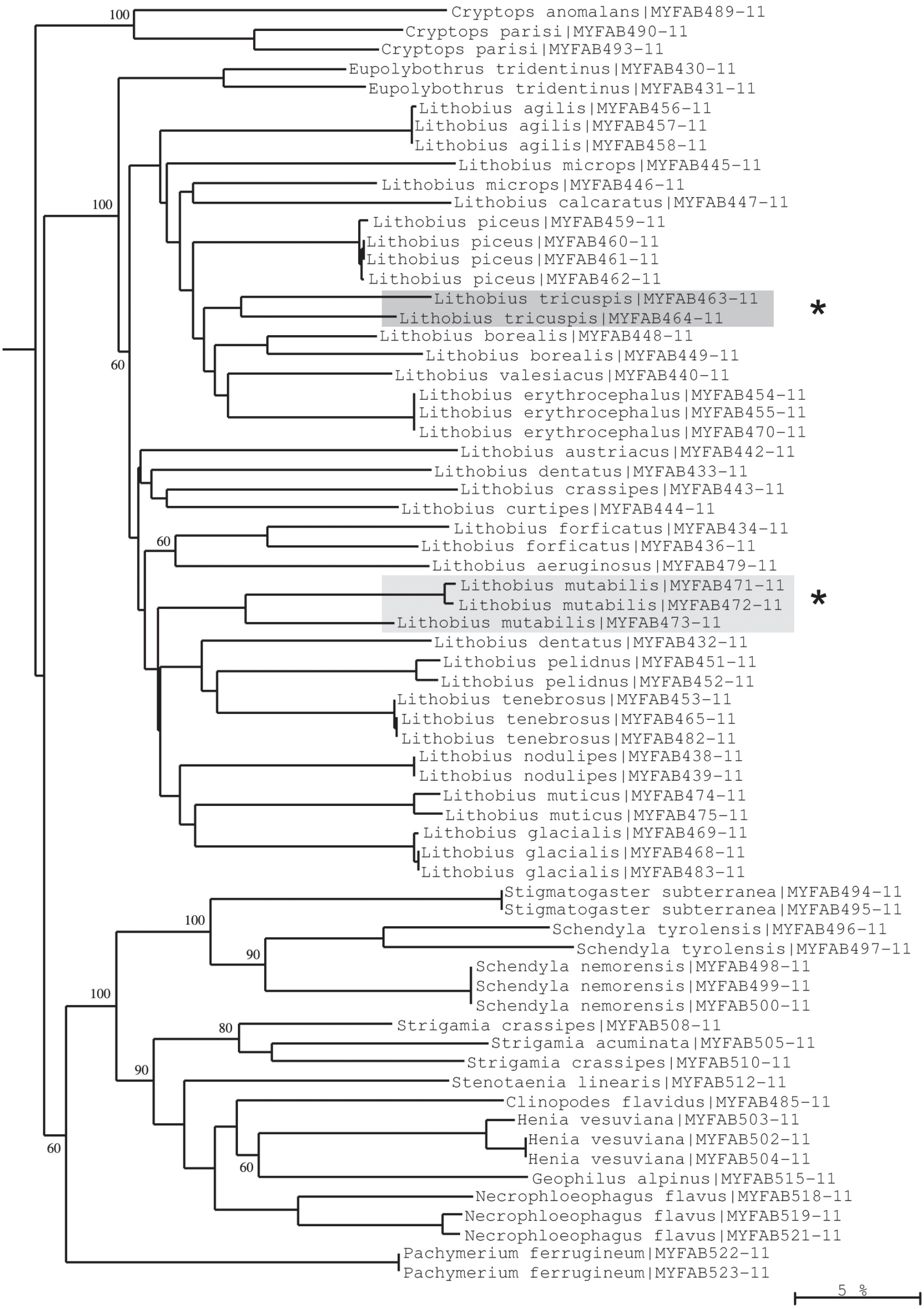

Figure 11. Neighbour-joining tree of COI sequence divergences (K2P model) of studied Chilopoda. Asterisks: Deep divergences within *Lithobius tricuspis* and *L. mutabilis* suggesting cryptic speciation. Numbers above and below branches show bootstrap values of neighbour-joining analysis, branch length indicates sequence divergence in %.

failed so far to get any sequences in the genera *Trachysphaera* and *Mycogona*, and have obtained only single chance results in the *Glomeris undulata* and the *Leptoiulus simplex* species groups. The genera *Ommatoiulus*, *Unciger* and *Tachypodoiulus* also seemed to be difficult, as we have obtained only a few barcodes for each of these taxa. To get optimal results special primers would have to be designed. But it is not only the primer design but also the protocol that influences the results. This might explain why some species yielded a barcode in one analytical plate but not in another. Contamination by chemicals (defense secretions in millipedes) might be another cause of unpredictable failures.

Although COI barcoding has provided an excellent tool for the identification of all life stages in several species, there are some problems with this gene locus as it is of mitochondrial origin. This means that it only shows maternal inheritance; therefore different maternal lines might mock cryptic species. This mainly affects the Chilopoda, which show a much higher genetic variability than the Diplopoda. While the histogram of intraspecific distances of the Diplopoda (Fig. 6) resembles that found in insects (e.g., Lepidoptera – Geometridae: Hausmann et al. 2011a), the histogram of the Chilopoda (Fig. 5) implies several undiscovered lineages, either of cryptic species or of long separated haplotypes.

Recent speciations of glacial or postglacial origin with ongoing hybridization and introgression are impossible to resolve using barcodes, as apparently shown by the genera *Craspedosoma*, *Rhymogona* and *Listrocheiritium*. In such cases other genes, especially of nuclear origin, should be used for evolutionary analysis in addition to COI.

Our results show that DNA barcoding can be a highly effective tool for the identification of Chilopoda and Diplopoda, provided that the right primers are designed and the right protocol is used. Before it can be better used, a reference barcode library is needed, the genetic variation must be known, and a close partnership between researchers with taxonomic expertise and those with a background in molecular analysis should be established for the interpretation of the results.

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